Neurogastroenterology and Motility

2006 Joint International Meeting

September 14 – 17, 2006

Seaport Hotel and World Trade Center
Boston, Massachusetts

Image compliments of the Greater Boston Convention & Visitors Bureau and the Massachusetts Convention Center Authority

Final Program

Jointly sponsored by

American Motility Society

European Society of Neurogastroenterology and Motility

Functional Brain-Gut Research Group

International Neurogastroenterology and Motility Group

University of Michigan Medical School

Hosted by the American Motility Society

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American Motility Society

European Society of Neurogastroenterology and Motility

International Neurogastroenterology and Motility Group

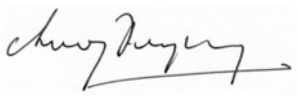
Functional Brain-Gut Research Group

WELCOME

It is with great pleasure that we welcome you to the 1st Joint International Neurogastroenterology and Motility Meeting. This is the inaugural meeting involving the memberships of the American Motility Society, the European Society of Neurogastroenterology and Motility, the Functional Brain-Gut Research Group, and the International Neurogastroenterology and Motility Group. The enthusiastic response from the sponsoring organizations to the announcement of this first meeting has been very gratifying. We believe this reflects the broad and growing interest in research involving the brain-gut axis and regulation of neuromuscular behavior in the gastrointestinal tract.

The organizing committees have worked diligently to assemble an outstanding scientific and social program that we believe will set the stage for successful future joint meetings. Please complete the continuing education evaluation to provide constructive feedback to the organizing committees. These comments will be reviewed carefully as the next meeting is being planned.

We all look forward to an exciting meeting in Boston.



Chung Owyang
Chair, Steering Committee

John Wiley
Chair, Program Committee

Henry Parkman
Co-Chair, Program Committee

SPEAKER-READY ROOM

If you submitted your presentation in advance, please visit the Speaker-Ready Room a minimum of 24 hours before your talk to preview it. Otherwise, if you have not pre-submitted your presentation, please see the AV technician 24 hours before your talk in the Plaza Meeting Room in the Seaport Hotel on Wednesday only and on Thursday-Sunday in the Tremont Room in the World Trade Center.

Upon arrival at the Speaker-Ready Room, a technician will check you in, upload your presentation if necessary, and then assign you to one of ten preview stations where you will be able to review and finalize your presentation. Internet connectivity is not available in oral session rooms.

The hours of operation are as follows:

Wednesday, September 13	12:00 p.m. – 6:00 p.m.
Thursday, September 14	7:00 a.m. – 6:00 p.m.
Friday, September 15	7:00 a.m. – 6:00 p.m.
Saturday, September 16	7:00 a.m. – 6:00 p.m.
Sunday, September 17	7:00 a.m. – 11:00 a.m.

POSTER SET UP

Location: Exhibition Hall – Seaport World Trade Center
Mount: After 5:00 p.m. on Wednesday, September 13 or by 10:30 a.m. on Thursday, September 14
Dismount: Between 3:30 and 5:30 p.m. on Saturday, September 16

CME CERTIFICATE INFORMATION
Your meeting evaluation form can be found in your registration packet. It must be completed and returned to the registration desk at **noon on Sunday, September 17**. At that time, you will be asked for your signature acknowledging your attendance and you will receive your certificate. Certificates will **NOT** be mailed to meeting participants. They are available only on-site.

EXHIBITOR HOURS
Thursday (optional), Friday and Saturday – 8:00 am – 5:00 pm in the Exhibition Hall

SOCIAL EVENTS
Thursday, September 14 Welcome Reception from 6:00 – 8:00 pm in the Plaza Ballroom, Seaport Hotel

Friday, September 15 Dinner Cruise on Boston Harbor – meet in the lobby of the Seaport Hotel at 6:15 pm to walk to the Spirit of Boston dock. Board the boat at 6:30 pm; Cruise from 7:00 – 10:00 pm. **You must bring your boarding pass and drink ticket with you to board the boat. Both items will be found in the back of your name tag. If you ordered extra cruise tickets, they will be included in your name tag.**

Saturday, September 16 Banquet at the Harvard Club of Boston – meet in the lobby of the Seaport Hotel at 6:15 pm to board the trolley to the Harvard Club. The banquet is from 7:00–10:00. Trolleys will return guests to the hotel. **Your banquet ticket will be in the back of your name tag. You will be asked for the ticket upon boarding the trolley. If you ordered extra banquet tickets, they will be included in your name tag.**

MEETING OBJECTIVES
The program has been designed to be timely, exciting, and informative. Upon completion of this meeting, attendees will have been exposed to state-of-the-art research covering a broad range of basic science, preclinical, and clinical topics in the rapidly expanding field of neurogastroenterology, gastrointestinal motility, and functional gastrointestinal disorders. These objectives will be achieved by a combination of lectures, oral abstract presentations, poster abstract presentations, small group discussions, and one-on-one interactions with recognized experts.

INTENDED AUDIENCE
This meeting will be valuable to a broad range of attendees – biochemists, molecular and cell biologists, physiologists, neurophysiologists, immunologists, pharmacologists, behavioral psychologists and psychiatrists, gastroenterologists and other clinicians, and gastroenterology fellows and nurses. The meeting promises a good interaction and exchange of ideas among participants, especially young investigators.

PROGRAM AT-A-GLANCE

Neurogastroenterology and Motility 2006 Joint International Meeting
Seaport Hotel and World Trade Center
Boston, Massachusetts

September 13–17, 2006

TIME	ACTIVITY AND LOCATION
	WEDNESDAY, SEPTEMBER 13, 2006
4:00 pm – 6:00 pm	**Registration** Seaport Hotel Lobby
12:00 pm – 6:00 pm	**Speaker-Ready Room** Plaza Meeting Room Seaport Hotel
	THURSDAY, SEPTEMBER 14, 2006
7:00 am – 6:00 pm **7:00 am – 8:00 am**	**Registration** Seaport Hotel Lobby **Continental Breakfast for Workshop Attendees** Harborview 2 – World Trade Center
7:00 am – 6:00 pm	**Speaker-Ready Room** Tremont Room – World Trade Center
8:00 am – 12:00 pm	**Workshop** Recent Advances in Understanding the Role of Serotonin in Gastrointestinal Motility and Functional Bowel Disorders Cityview 1 – World Trade Center
8:00 am – 12:00 pm *7:15 am bus departs*	**Workshop** Brain Imaging and Neurogastroenterology *Held off-site* Martinos Center at Massachusetts General Hospital Conference Room A 149 Thirteenth Street Charlestown, Massachusetts (*need to purchase bus ticket for $10*)
12:00 pm – 1:00 pm	**Lunch for Workshop Attendees** Harborview 2 – World Trade Center
1:00 pm – 5:00 pm	**Workshop For Young Investigators** Experimental Approaches to Understanding the Enteric Nervous System in Health and Disease Cityview 1 – World Trade Center
1:00 pm – 5:00 pm	**Workshop** Consensus Guidelines for Gastric Emptying Scintigraphy Cityview 2 – World Trade Center

1:30 pm – 5:00 pm	**Workshop** Understanding Placebos in Clinical Practice and Trials Back Bay Complex – World Trade Center
6:00 pm – 8:00 pm	**Welcome Reception** Plaza Ballroom – Seaport Hotel
	FRIDAY, SEPTEMBER 15, 2006
7:30 am – 5:00 pm **7:00 am – 7:45 am**	**Registration** Atrium Lobby – World Trade Center **Continental Breakfast** Cityview Ballroom
7:00 am – 6:00 pm	**Speaker-Ready Room** Tremont Room – World Trade Center
7:45 am – 12:05 pm	**Opening Plenary Session** New Concepts in Neurogastroenterology: Cell Mechanisms and Integrated Systems Harborview Ballroom
12:05 pm – 1:30 pm	**2008 Scientific Planning Committee Meeting** Skyline Room *(invited attendees only)*
12:05 pm – 3:00 pm	**Lunch and Poster Session** Exhibition Hall – World Trade Center
3:00 pm – 5:00 pm	**Concurrent Sessions** Electrical Stimulation and Pacing in the GI Tract Harborview Ballroom Novel Molecular Targets and Their Role in GI Symptom Amphitheater GI Motility Disorders in Children and Adolescents Waterfront Ballroom
5:00 pm – 5:30 pm	**American Motility Society Business Meeting** Amphitheater
5:00 pm – 6:00 pm	**European Society of Neurogastroenterology and Motility General Assembly** Harborview Ballroom
6:15 pm **6:30 pm – 10:00 pm**	**Meet in the Seaport Hotel Lobby with Boarding Pass and Drink Ticket** **Dinner Cruise on Boston Harbor**
	SATURDAY, SEPTEMBER 16, 2006
6:00 am – 7:45 am	**Elective Breakfast Symposium** New Mechanisms and Novel Concepts in Chronic Constipation *Sponsored by Sucampo Pharmaceuticals, Inc. and Takeda Pharmaceuticals North America* Waterfront Ballroom
7:30 am – 5:00 pm **7:00 am – 8:00 am**	**Registration** Atrium Lobby – World Trade Center **Continental Breakfast** Cityview Ballroom

7:00 am – 8:00 am	**All Society Committee Meeting** Skyline Room *(invited attendees only)*
7:00 am – 6:00 pm	**Speaker-Ready Room** Tremont Room – World Trade Center
8:00 am – 12:15 pm	**Plenary Session** The Role of Immune Modulation in the Brain-Gut Axis Novel Therapeutic Management of Gastrointestinal Motility and Functional Gastrointestinal Disorders Harborview Ballroom
12:15 pm – 3:00 pm	**Lunch and Poster Session** Exhibition Hall – World Trade Center
1:00 pm – 3:00 pm	**FBG Board Meeting** Washington Room – World Trade Center *(invited attendees only)*
3:00 pm – 5:00 pm	**Concurrent Sessions** Novel Signaling Pathways Harborview Ballroom Novel Approaches to Diagnosis Amphitheater Methodologies For Health Care Research Waterfront Ballroom
6:15 pm **6:30 pm – 10:00 pm**	**Meet in the Seaport Hotel Lobby for Trolley with Banquet Ticket** **Banquet at the Harvard Club of Boston**
	SUNDAY, SEPTEMBER 17, 2006
7:30 am – 1:00 pm **7:00 am – 8:00 am**	**Registration** Atrium Lobby – World Trade Center **Continental Breakfast** Cityview Ballroom
7:00 am – 11:00 am	**Speaker-Ready Room** Tremont Room – World Trade Center
8:00 am – 12:00 pm	**Plenary Session** Regulation of Appetite and Obesity: Where Are We Now and Where Are We Headed? **Keynote Speakers:** Models of Metabolism: Of Mice and Men Charles Burant, *University of Michigan Health System* Modeling Complex Biological Systems: Where Are We Headed? Leroy Hood, *Institute for Systems Biology* Harborview Ballroom
1:00 pm – 5:00 pm	**Workshop** Cyclic Vomiting Syndrome in Adults Back Bay Complex – World Trade Center

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ACKNOWLEDGMENTS

The American Motility Society, the European Society of Neurogastroenterology and Motility, the Functional Brain-Gut Research Group, and the International Neurogastroenterology and Motility Group gratefully acknowledge the generous educational support received from the following companies:

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Andrea M. Harrington
Sebastian Hoff
Sumei Liu
Bridget R. Southwell
Burkhard Stoffels
José J. ter Linde
Maria Vazquez Roque

Young Investigator Workshop Award Recipients

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Michael J. Beyak
Xiaochun Bian
Arun Chaudhury
John O. Clarke
Heiko U. De Schepper
Michael P. Frese
Patrick A. Hughes
Andrea Lorincz
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Nikhil Thapar
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Olga I. Santiago-Maldonado

Abstracts of Distinction

(Submitted abstracts scored in the top 10%)

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Christopher N. Andrews
Caroline Appleyard
Andrea Balletta
Rebecca Bertrand
Ashley Blackshaw
Simon Brookes
Cristina Camello-Almaraz
Bindu Chandrasekharan
Elizabeth Colley
Joseph Davison
Roberto De Giorgio
Benedicte De Winter
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John Johanson
Eric Krauter
Alfons Kroese
Muriel Larauche
Ying Li
David Linden
Mintsai Liu
Sumei Liu
William Paterson
Elyanne Ratcliffe
José M. Remes-Troche
Giovanni Sarnelli
Michael Schemann
Joachim Schmidt
Lisa Schmidt
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Terence Smith
Bridget R. Southwell
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Maria Vazquez Roque
John Winston
Xiaohong Xu
Jing Zhang

ACCREDITATION STATEMENT

This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the University of Michigan Medical School and the American Motility Society. The University of Michigan Medical School is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

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Anurag Agrawal is a speaker for and receives grant support from Novartis Pharmaceuticals.

Christopher Andrews is a consultant for and received grant/research support from Johnson and Johnson.

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Alfred Bayati is an employee of Astra Zeneca.

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Simon Brookes receives grant/research support from AstraZeneca.

Michael Camilleri is a consultant for Mayo Clinic Alliance with Enteromedics Co. and Novartis.

Lin Chang is a consultant for GSK, Microbia and Vela and receives grant support from GSK.

Elizabeth Colley is a shareholder of Holburn.

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Beverley Greenwood-Van Meerveld receives grant support from Novartis.

Denis Guyonnet is a consultant for and receives grant/research support from Danone Research.

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John Johanson is a consultant for Sucampo Pharmaceuticals, Inc., Takeda Pharmaceuticals North America, Inc., Boehringer Ingelheim Corporation, Prometheus Laboratories, Inc., and Theravance, Inc., a speaker for Takeda Pharmaceuticals North America, Inc. and Novartis Pharmaceutical Corporation, and has other financial interest in Sucampo Pharmaceuticals, Inc.

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Michael A Kamm is a consultant for Medtronic and receives grant support from Medtronic.

Suzanne Kelly is an employee of Novartis Pharmaceuticals, Canada Inc.

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Kirsten Tillisch receives grant/research support from Avera.

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Husatyju Uneyama is a stockholder in Ajinomoto Co., Inc.

Sander van Zanten is a consultant for Novartis and Astellas.

William Whitehead is a consultant for Microbia Pharmaceuticals, GSK Pharmaceuticals, Bristol Meyers Pharmaceuticals, Takeda Pharmaceuticals, Sucampo Pharmaceuticals is a speaker for Takeda Pharmaceuticals, Novartis Pharmaceuticals, and receives grant/research support from Novartis Pharmaceuticals.

John Wiley receives grant/research support from Novartis Pharmaceutical.

Jackie Wood is a speaker for Norvartis Pharmaceuticals Inc. and receives grant support from Sucampo Pharmaceuticals Inc.

Mira Wouters receives grant/research support from Solvay Pharmaceuticals.

Shaoyong Yu receives grant/research support from AstraZeneca.

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The Ohio State University College of Medicine

Exhibitor Floor Plan
2006 Neurogastroenterology and Motility Meeting

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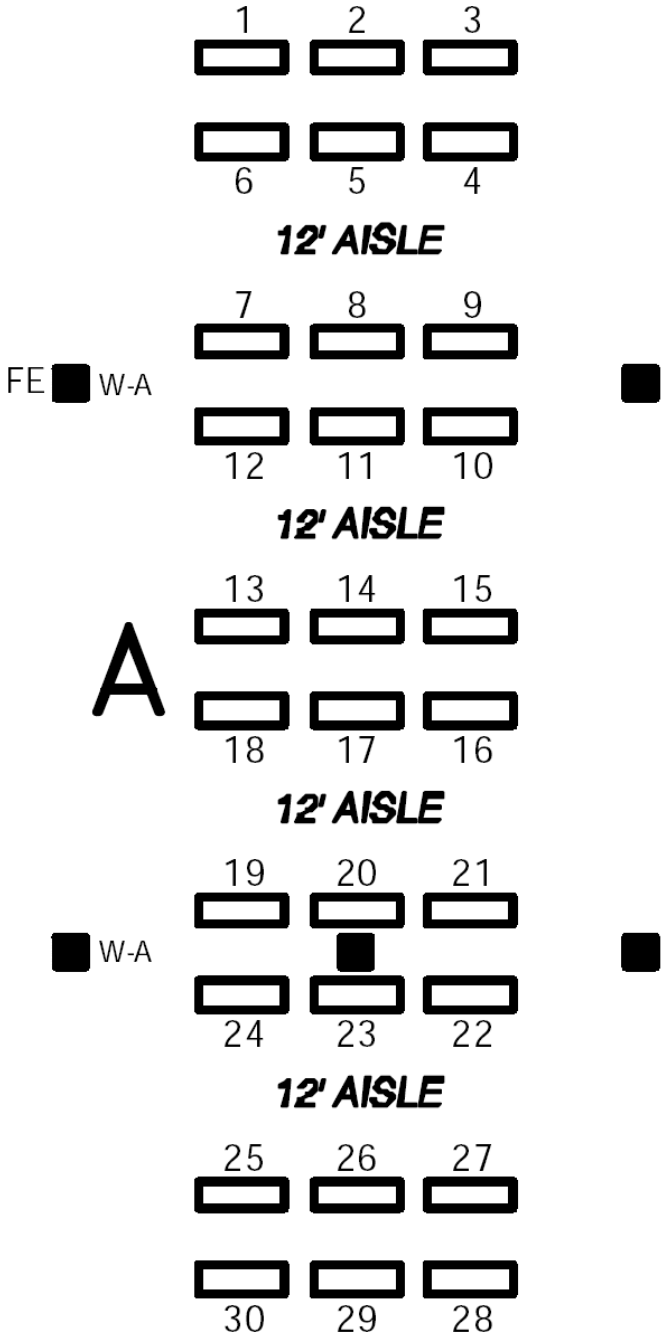

WORKSHOPS

WORKSHOP
RECENT ADVANCES IN UNDERSTANDING THE ROLE OF SEROTONIN IN GASTROINTESTINAL MOTILITY AND FUNCTIONAL BOWEL DISORDERS

Thursday, September 14, 2006
8:00 AM – 12:00 PM
Cityview 1 – World Trade Center

James Galligan and Henry Parkman, Presiding

8:00 INTRODUCTION

DISTRIBUTION AND FUNCTION OF 5-HT RECEPTORS

8:15 5-HT RECEPTORS ON ENTERIC NEURONS AND SMOOTH MUSCLE: STUDIES IN HUMANS.
Michael Schemann, *Technical University Munich*

8:45 IMPORTANCE OF 5-HT RECEPTORS ON INTESTINAL AFFERENTS IN THE REGULATION OF VISCERAL SENSITIVITY.
Beverly Greenwood van Meerveld, *University of Oklahoma*

9:15 5-HT RECEPTORS AND INTERSTITIAL CELLS OF CAJAL (ICCs).
Gianrico Farrugia, *Mayo Clinic*

9:45 am – 10:00 am Intermission

SEROTONIN IN GI MOTILITY AND FUNCTIONAL BOWEL DISORDERS

William Hasler, Presiding

10:00 ALTERATIONS IN 5-HT SIGNALING AND METABOLISM IN HUMAN DISEASE.
Robin Spiller, *University Hospital*

10:30 MECHANISMS OF SEROTONERGIC AGENTS FOR TREATMENT OF GI MOTILITY AND FUNCTIONAL BOWEL DISORDERS ($5\text{-}HT_1$, $5\text{-}HT_2$, $5\text{-}HT_3$, $5\text{-}HT_4$).
Jan Tack, *University Hospitals Leuven*

11: 00 PHARMACOGENOMICS AND SEROTONIN: RESEARCH AND CLINICAL PRACTICE IMPLICATIONS.
Michael Camilleri, *Mayo Clinic*

11: 30 FUTURE DIRECTIONS IN UNDERSTANDING THE ROLE OF SEROTONIN IN GI MOTILITY AND FUNCTIONAL BOWEL DISORDERS. OVERVIEW AND DISCUSSION.
Michael Gershon, *Columbia University*

12:00 ADJOURN

This activity is supported by an educational grant from Novartis Pharmaceuticals

WORKSHOP
FUNCTIONAL IMAGING IN GASTROENTEROLOGY

Thursday, September 14, 2006
8:00 AM – 12:00 PM
Martinos Center at Massachusetts General Hospital
Conference Room A
149 Thirteenth Street
Charlestown, Massachusetts

Braden Kuo and Emeran Mayer, Presiding

The goal of this symposium is to update participants on the fundamentals and the recent advances in functional neuroimaging and its role in understanding brain-gut interactions in health and disease. Presentations will include reviews of basic imaging techniques, findings in health and disease, issues with imaging analysis, placebo response and future directions of imaging and gastroenterology. This symposium is geared to basic scientists, clinical investigators, academic gastroenterologists, young investigators including gastroenterology faculty, gastroenterology fellows, post-docs, and pharmaceutical scientists and representatives.

8:00 INTRODUCTION

8:05 SO YOU WANT TO DO AN IMAGING EXPERIMENT?
Braden Kuo, *Massachusetts General Hospital*

8:30 IMAGING OF GI SENSATION IN HEALTHY VOLUNTEERS.
Mark Kern, *Medical College of Wisconsin*

9:00 IMAGING IN FUNCTIONAL GI DISEASE.
Yehuda Ringel, *University of North Carolina*

9:30 NETWORK MODELS IN IRRITABLE BOWEL SYNDROME.
Jennifer Labus, *University of California, Los Angeles*

10:00 am – 10:15 am Intermission

10:15 APPLICATION OF TMS, MEG AND MRS IN EXPLORING CORTICAL CONTROL OF GI FUNCTION.
Shaheen Hamdy, *University of Manchester*

10:45 IMAGING THE PLACEBO RESPONSE.
Randy Gollub, *Massachusetts General Hospital*

11:15 FUTURE DIRECTIONS OF FUNCTIONAL IMAGING.
Bruce Rosen, *Massachusetts General Hospital*

11: 40 FUTURE DIRECTIONS IN FUNCTIONAL IMAGING AND GASTROENTEROLOGY.
Emeran Mayer, *University of California, Los Angeles*

12:00 LUNCH BREAK OR RETURN TO HOTEL

1:00 TOUR OF IMAGING FACILITIES
Animal imaging, standard MRI imaging, high-field 7T MRI, MEG

2:00 RETURN TO SEAPORT HOTEL

WORKSHOP FOR YOUNG INVESTIGATORS
EXPERIMENTAL APPROACHES TO UNDERSTANDING THE ENTERIC NERVOUS SYSTEM IN HEALTH AND DISEASE

Thursday, September 14, 2006
1:00 PM – 5:00 PM
Cityview 1 – World Trade Center

James Galligan and Fernando Azpiroz, Presiding

1:00 ANIMAL MODELS OF GI MOTILITY DISORDERS.
Beverley Greenwood Van Meerveld, *Oklahoma University*

1:30 ADVANCED TECHNIQUES FOR STUDIES OF GI MOTILITY *IN VITRO.*
Peter Holzer, *University of Graz*

2:00 ADVANCED IMAGING AND OTHER TECHNIQUES FOR GI MOTILITY STUDIES IN HUMANS.
Jan Tack, *University Hospitals Leuven*

2:30 ANIMAL MODELS OF VISCERAL PAIN.
Lionel Bueno, *Institut Nationale de la Recherche Agronomique*

3:00 pm – 3:30 pm Intermission

3:30 TECHNIQUES FOR ASSESSING VISCERAL PAIN MECHANISMS IN HUMANS.
Emeran Mayer, *David Geffen School of Medicine at UCLA*

4:00 IMAGING NEURONAL SIGNALING IN THE HUMAN ENS.
Michael Schemann, *Techniche Universitat Munich*

4:30 LASER CAPTURE AND SINGLE CELL TECHNIQUES FOR STUDIES OF mRNA EXPRESSION.
Catia Sternini, *David Geffen School of Medicine at UCLA*

5:00 ISOLATION AND CULTURE OF ICCS, SMOOTH MUSCLE AND ENTERIC NEURONS.
Gianrico Farrugia, *Mayo Clinic*

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WORKSHOP
CONSENSUS GUIDELINES FOR GASTRIC EMPTYING SCINTIGRAPHY
A Joint Project of the Society of Nuclear Medicine and the American Motility Society

Thursday, September 14, 2006
1:00 PM – 5:00 PM
Cityview 2 – World Trade Center

Henry Parkman and Alan Maurer, Presiding

1:00 INTRODUCTION AND OVERVIEW
Henry Parkman, *Temple University*

1:05 ISSUES FOR PATIENTS.
Jeanne Keith-Ferris, *Gastroparesis and Dysmotility Association*

1:15 WHAT THE CLINICIAN WANTS TO KNOW IN A GASTRIC EMPTYING TEST.
William Snape, *California Pacific Medical Center,* Larry Szarka, *Mayo Clinic, and Tom Nowak, Saint Vincent Hospital*

1:45 WHAT NUCLEAR MEDICINE PROVIDES IN A GASTRIC EMPTYING TEST.
Academic Medicine: Harvey Zeissman, *Johns Hopkins Hospital* / **Private Practice:** Paul Shreve

2:10 GASTRIC EMPTYING FOR GASTROPARESIS: HISTORY AND DEVELOPMENT OF THE LOW FAT MEAL.
Richard McCallum, *Kansas University Medical Center*

2:30 ASSESSING RAPID GASTRIC EMPTYING WITH THE LOW FAT MEAL.
Tom Abell, *University of Mississippi*

2:45 GASTRIC EMPTYING SCINTIGRAPHY AND CLINICAL RESEARCH.
William Hasler, *University of Michigan Health System*

3:00 pm – 3:20 pm Intermission

3:20 PROPOSAL FOR STANDARDIZATION OF GASTRIC EMPTYING – DISCUSSION
Alan Maurer, *Temple University*

3:50 GASTRIC EMPTYING SCINTIGRAPHY GUIDELINES 1.0.
Kevin Donohoe, *Beth Israel Deaconess Medical Center* and Henry Parkman, *Temple University*

4:20 PATIENT AND CLINICAL INFORMATION FOR GASTRIC EMPTYING SCINTIGRAPHY.
Henry Parkman, *Temple University*

4:35 ADVANCED TECHNIQUES FOR GASTRIC EMPTYING SCINTIGRAPHY.
Alan Maurer, *Temple University*

4:50 ADVANCED ANALYSIS FOR GASTRIC EMPTYING SCINTIGRAPHY.
Martin Nusynowitz, *University of Texas Medical Branch*

5:00 IS THERE A NEED TO DEVELOP A SECOND STANDARDIZED MEAL?
Kevin Donohoe, *Beth Israel Deaconess Medical Center*

This activity is supported by an educational grant from Novartis and TAP Pharmaceuticals and the Gastroparesis and Dysmotility Association

WORKSHOP
UNDERSTANDING PLACEBOS IN CLINICAL PRACTICE AND TRIALS

Thursday, September 14, 2006
1:30 PM – 5:00 PM
Back Bay Complex – World Trade Center

Paul Enck and Tony Lembo, Presiding

1:30 PLACEBO – FOES OR FRIENDS IN MEDICINE?
W. Grant Thompson, *University of Ottawa*

2:00 PLACEBOS, ACUPUNCTURE, AND ALTERNATIVE MEDICINE.
Ted Kaptchuk, *Harvard University*

2:30 EXPERIMENTAL PLACEBO RESEARCH
Sibylle Klosterhalfen, *University of Düesseldorf*
Paul Enck, *University of Tüebingen*

3:00 pm – 3:30 pm Intermission

3:30 IMAGING THE PLACEBO RESPONSE IN THE BRAIN.
Emeran Mayer, *David Geffen School of Medicine at UCLA*

4:00 PLACEBO EFFECTS IN IBS CLINICAL TRIALS.
Tony Lembo, *Harvard University*

4:30 INNOVATIVE CLINICAL TRIAL DESIGNS.
Paul Enck, *University of Tüebingen*
Sibylle Klosterhalfen, *University of Düesseldorf*

Supported by an educational grant from Holburn Biomedical Corporation

ELECTIVE BREAKFAST SYMPOSIUM
NEW MECHANISMS AND NOVEL CONCEPTS IN CHRONIC CONSTIPATION

Saturday, September 16, 2006
6:00 AM – 7:45 AM
Waterfront Ballroom – World Trade Center

Registration – Note that this symposium requires a separate registration.

5:45 REGISTRATION ON-SITE IN THE WATERFRONT BALLROOM. BREAKFAST WILL BE SERVED.

6:00 INTRODUCTION
Lin Chang, Faculty Chair
David Geffen School of Medicine at UCLA

6:05 MECHANISMS UNDERLYING CHRONIC CONSTIPATION.
Michael A. Kamm
St. Mark's Hospital, Harrow, England

6:30 THE UTILITY OF DIAGNOSTIC TESTS FOR CHRONIC CONSTIPATION.
Satish S.C. Rao
University of Iowa

6:45 THE MECHANISMS BEHIND THE TREATMENT.
Lin Chang
David Geffen School of Medicine at UCLA

7:05 CURRENT AND EMERGING THERAPIES FOR CHRONIC CONSTIPATION.
William D. Chey
University of Michigan Medical Center

Satish S.C. Rao
University of Iowa

7:30 QUESTION-AND-ANSWER SESSION

Jointly sponsored by the Dannemiller Memorial Educational Foundation and The Customer Link, Inc. This activity is supported by an educational grant from Sucampo Pharmaceuticals, Inc. and Takeda Pharmaceuticals North America.

CME Accreditation
This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (AACME) through the joint sponsorship of the Dannemiller Memorial Educational Foundation and The Customer Link, Inc. The Dannemiller Memorial Educational Foundation is accredited by the Accreditation Council for Continuing Medical Education (AACME) to provide continuing medical education for physicians.

The Dannemiller Memorial Educational Foundation designates this educational activity for a maximum of 1.75 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

WORKSHOP
CYCLIC VOMITING SYNDROME IN ADULTS

Sunday, **September 17, 2006**
1:00 pm – 5:00 pm
Back Bay Complex – World Trade Center

B Li, Henry Parkman, and Richard McCallum, Presiding

1:00 INTRODUCTION

1:05 CLINICAL PRESENTATION OF CVS IN ADULTS.

Clinical Symptoms and Epidemiology of CVS in Adults
David R. Fleisher, *University of Missouri Hospital and Clinics*

Natural History of CVS: Pediatric to Adulthood
Athos Bousvaros, *Children's Hospital of Boston, Harvard Medical School*

1:35 CLINICAL SPECTRUM OF CVS IN ADULTS.

Co-morbidities and Complications – Henry Parkman, *Temple University*
Diagnostic Testing – Richard McCallum, *Kansas University Medical Center*

2:05 THERAPEUTIC ADVENTURES AND FRONTIERS IN CVS.

A**cute Treatment** – Tom Abell, *University of Mississippi Medical Center*
Prophylactic Treatment – Kevin Olden, *University of Arkansas for Medical Sciences*

2:35 REVIEW AND CRITIQUE OF CLINICAL ASPECTS OF CVS IN ADULTS.

Panel Discussion
Sonia Friedman, *Brigham and Women's Hospital /* William Hasler, *University of Michigan Health System* / Nimish Vakil, *Medical College Wisconsin*

3:00 pm – 3:20 pm Intermission

3:20 NEW CONCEPTS ON THE PATHOGENESIS AND TREATMENT OF CVS.

CRF, Stress, and the Brain-Gut / Hypothalamic-Pituitary Axis
Yvette Taché, *David Geffen School of Medicine at UCLA*

Mitochondria and Maternally-Inherited Dysautonomia
B. Li, *Medical College of Wisconsin*

Role of Brain Imaging and Lessons from Migraine
Emeran Mayer, *David Geffen School of Medicine at UCLA*

4:20 CVS IN ADULTS: WHERE RESEARCH IS NEEDED

Development of Definitions and Registry for CVS in Adults.
Nimish Vakil and B. Li, *Medical College of Wisconsin*

WHERE DO WE GO FROM HERE?
Henry Parkman, B. Li, Richard McCallum, and Kevin Olden

5:00 ADJOURN

Co-sponsored by the Cyclic Vomiting Syndrome Association, Dynogen, and the American Motility Society

SCIENTIFIC SESSIONS

OPENING PLENARY SESSION

FRIDAY, SEPTEMBER 15, 2006
7:45 AM – 10:00 AM
Harborview Ballroom

7:45 INTRODUCTION
Chung Owyang and Fernando Azpiroz

NEW CONCEPTS IN NEUROGASTROENTEROLOGY: CELL MECHANISMS

David Grundy and Lesley Houghton, Presiding

8:00 ENTERIC NERVES AND GLIAL CELLS IN HEALTH AND DISEASE.
Simon JH Brookes, *Flinders University*

8:25 HYDROGEN SULFIDE: MODE OF ACTION OF A NOVEL NEUROMODULATOR IN THE HUMAN AND GUINEA-PIG GUT. *M Schemann*, R Schicho, D Krueger, CW Hann von Weyhern, F Zeller, T Frieling, H Kimura, I Ishii, R DeGiorgio, and B Campi, Freising, Munich, and Krefeld, Germany, Tokio and Gunma, Japan, Bologna and Ferrara, Italy. *Technical University* ***(Abstract of Distinction)*** Abstract 1

8:40 INTERSTITIAL CELLS OF CAJAL IN HEALTH AND DISEASE.
Gianrico Farrugia*, Mayo Clinic College of Medicine*

9:05 IDENTIFICATION OF MECHANO-NOCICEPTOR ENDINGS IN THE GASTROINTESTINAL TRACT. *SJ Brookes*, XY Song, BN Chen, and M Costa, Bedford Park, and Adelaide, SA Australia. *Flinders University* ***(Abstract of Distinction)*** Abstract 2

9:20 SMOOTH MUSCLE IN HEALTH AND DISEASE.
Karnam Murthy, *Virginia Commonwealth University*

9:45 CA^{2+} SENSITIZATION-MEDIATED CONTRACTION IN AGED GUINEA PIG GALLBLADDER. *C Camello-Almaraz*, MJ Pozo, B Macias, PJ Camello, PJ Gomez-Pinilla, and R Moreno, Cácere, Spain *University of Extremadura* ***(Abstract of Distinction)*** Abstract 3

10:00 am – 10:15 am Intermission

NEW CONCEPTS IN NEUROGASTROENTEROLOGY: INTEGRATED SYSTEMS

10:15 AM – 12:05 PM
Harborview Ballroom

Ann Ouyang and Henry Parkman, Presiding

10:15 VISCERAL PERCEPTION: AFFERENT PATHWAYS.
Gerald F. Gebhart*, University of Iowa*

10:40 NODOSE GANGLIA NEURONS DEMONSTRATE GLUCOSE-EXCITATORY AND GLUCOSE-INHIBITORY RESPONSES MEDIATED VIA POTASSIUM CHANNELS.
G Grabauskas, SY Zhou, and C Owyang, Ann Arbor, MI. *University of Michigan* ***(Young Investigator Travel Awardee and Abstract of Distinction)*** Abstract 4

10:55 VISCERAL PERCEPTION: THE ROLE OF ENDOGENOUS MODULATION.
Emeran Mayer, *David Geffen School of Medicine at UCLA*

11:20 OCCULT REFLEXES UNDERLYING COLONIC STORAGE ARE ACTIVATED BY INTRINSIC NEURONS RESPONDING TO LONGITUDINAL STRETCH.
TK Smith, EJ Dickson, GW Hennig, and NJ Spencer, Reno, NV. *University of Nevada* ***(Abstract of Distinction)*** Abstract 5

11:35 VISCERAL PERCEPTION: COGNITIVE AND PSYCHOLOGICAL MECHANISMS.
William Whitehead, *University of North Carolina at Chapel Hill*

11:50 A NEW METHODOLOGY TO IDENTIFY CENTRAL PROCESSES INVOLVED IN VISCERAL PAIN PROCESSING. *B Greenwood-Van Meerveld*, R Towner, Y Tesiram, J Lazovic, B Myers, and AC Johnson, Oklahoma City, OK and Pasadena, CA. *University of Oklahoma Health Sciences Center* ***(Abstract of Distinction)*** Abstract 6

LUNCH AND POSTER SESSION
12:05 PM – 3:00 PM
Seaport World Trade Center Exhibition Hall

CONCURRENT SESSIONS

ELECTRICAL STIMULATION AND PACING IN THE GI TRACT

One of Three Concurrent Sessions
3:00 PM – 5:00 PM
Harborview Ballroom

Jay Huizinga and Richard McCallum, Presiding

3:00 INTERSTITIAL CELLS OF CAJAL: PHARMACOLOGY AND ROLE IN INTRINSIC PACING.
Kenton Sanders, *University Nevada*

3:25 TEMPORARY PERCUTANEOUS GASTRIC ELECTRICAL STIMULATION (GES). A NEW TECHNIQUE TO TEST RESPONSE TO GES. *H Abrahamsson*, A Elfvin, G Ringstrom, S Andersson, M Simrén, and H Lönroth, Gothenburg, Sweden. *Sahlgrenska University Hospital* ***(Abstract of Distinction)*** Abstract 7

3:40 GASTRIC ELECTRICAL STIMULATION AND PACING: MECHANISMS, PARAMETERS, RESULTS.
Jiande Chen, *University of Texas Medical Branch*

4:05 CENTRAL NEURONAL MECHANISMS OF GASTRIC ELECTRICAL STIMULATION AND ALTERATIONS IN CENTRAL NEURONAL ACTIVITIES IN OBESE RATS.

J Zhang and JD Chen, Oklahoma City, OK and Galveston, TX. *Veterans Research Foundation, VA Medical Center* ***(Abstract of Distinction)*** Abstract 8

4:20 COLORECTAL STIMULATION: WHO, HOW, AND WHY?
Michael Kamm, *St. Mark's Hospital*

4:45 INHIBITORY EFFECTS OF ELECTRICAL STIMULATION OF THE STOMACH, INTESTINE AND COLON ON RECTAL TONE. *XH Xu*, S Liu, and JD Chen, Oklahoma City, OK and Galveston, TX. *Veterans Research Foundation, VA Medical Center* ***(Abstract of Distinction)*** Abstract 9

5:00 AMERICAN MOTILITY SOCIETY BUSINESS MEETING – Amphitheater
5:00 ESNM GENERAL ASSEMBLY – Harboview Ballroom
6:30 DINNER CRUISE ON BOSTON HARBOR – Meet in the Seaport hotel lobby at 6:15 pm with boarding pass and drink ticket.

NOVEL MOLECULAR TARGETS AND THEIR ROLE IN GI SYMPTOMS

One of Three Concurrent Sessions
3:00 PM – 5:00 PM
Amphitheater

Charalabos Pothoulakis and Chung Owyang, Presiding

3:00 CANNABINOID RECEPTORS.
Keith Sharkey*, University of Calgary*

3:25 EFFECT OF A CANNABINOID RECEPTOR AGONIST ON HUMAN COLONIC MOTOR AND SENSORY FUNCTIONS. *T Esfandyari*, I Ferber, D Burton, K Baxter, AR Zinsmeister, and M Camilleri, Rochester, MN. *Mayo Clinic College of Medicine* ***(Abstract of Distinction)*** Abstract 10

3:40 PROTEASE ACTIVATED RECEPTORS.
Lionel Bueno*, National Institute of Agronomic Research/ Institut National de la Recherche Agronomique*

4:05 PROTEINASE-ACTIVATED RECEPTOR-2 (PAR-2) ACTIVATION EVOKES ESOPHAGEAL LONGITUDINAL SMOOTH MUSCLE (LSM) CONTRACTION VIA A CAPSAICIN-SENSITIVE AND NEUROKININ-2 (NK_2) RECEPTOR-DEPENDENT PATHWAY. *WG Paterson*, H Liu, DV Miller, S Lourenssen, RW Wells, and MG Blennerhassett, Kingston, Ontario, Canada. *Queen's University* ***(Abstract of Distinction)*** Abstract 11

4:20 AUTOIMMUNE MECHANISMS IN AUTONOMIC NEUROPATHY.
Vanda Lennon, *Mayo Clinic College of Medicine*

4:45 SENSORY-MOTOR ABNORMALITIES IN SEVERE GUT DYSMOTILITY: ROLE OF ANTI-HuD ANTI-NEURONAL ANTIBODY (A-HuD). *R De Giorgio*, L Talamonti, Q Li, M Beyak, M Trevisani, K Michel, B Campi, G Barbara, V Stanghellini, R Corinaldesi, P Geppetti, D Grundy, and M Schemann, Bologna and Ferrara, Italy, Munich and Freising, Germany,

Kingston, Ontario, Canada, and Sheffield, United Kingdom. *University of Bologna* ***(Abstract of Distinction)*** Abstract 12

5:00 AMERICAN MOTILITY SOCIETY BUSINESS MEETING – Amphitheater
5:00 ESNM GENERAL ASSEMBLY – Harboview Ballroom
6:30 DINNER CRUISE ON BOSTON HARBOR – Meet in the Seaport hotel lobby at 6:15 pm with boarding pass and drink ticket.

GI MOTILITY DISORDERS IN CHILDREN AND ADOLESCENTS

One of Three Concurrent Sessions
3:00 PM – 5:00 PM
Waterfront Ballroom

Carlo DiLorenzo and Samuel Nurko, Presiding

3:00 CONSTIPATION.
Marc A. Benninga, *Emma Children's Hospital, AMC*

3:25 TRANSIT STUDIES, COLONIC MANOMETRY AND TRANSCUTANEOUS ELECTRICAL STIMULATION TO DIAGNOSE AND TREAT SLOW TRANSIT CONSTIPATION IN CHILDREN. *BR Southwell*, JR Sutcliffe, SK King, J Chase, VJ Robertson, D Cook, A Catto-Smith, S Gibbs, and JM Hutson, Parkville, Victoria, and Melbourne, Australia. *Murdoch Childrens Research Institute and Royal Children's Hospital* ***(Young Investigator Travel Awardee and Abstract of Distinction)*** Abstract 13

3:40 CYCLIC VOMITING SYNDROME.
B U.K. Li, *Medical College of Wisconsin*

4:05 THE INCIDENCE OF CYCLICAL VOMITING SYNDROME IN THE PAEDIATRIC POPULATION OF IRELAND. *EC Fitzpatrick*, M Rowland, M Sherlock, B Drumm, and B Bourke, Dublin, Ireland. *University College Dublin* ***(Abstract of Distinction)*** Abstract 14

4:20 CHILDREN WITH FUNCTIONAL GI DISORDERS: SHORT TERM AND LONG TERM CLINICAL OUTCOMES.
Lynn S. Walker, *Vanderbilt University*

4:45 IS VISCERAL HYPERALGESIA CORRELATED WITH SYMPTOMS SEVERITY IN CHILDREN WITH FUNCTIONAL GASTROINTESTINAL DISORDERS? *C Faure*, J Castilloux, and A Noble, Montreal, Quebec, Canada. *Ste-Justine Hospital* ***(Abstract of Distinction)*** Abstract 15

5:00 AMERICAN MOTILITY SOCIETY BUSINESS MEETING – Amphitheater
5:00 ESNM GENERAL ASSEMBLY – Harboview Ballroom
6:30 DINNER CRUISE ON BOSTON HARBOR – Meet in the Seaport hotel lobby at 6:15 pm with boarding pass and drink ticket.

PLENARY SESSSION

SATURDAY, SEPTEMBER 16, 2006
8:00 AM – 10:00 AM
Harborview Ballroom

THE ROLE OF IMMUNE MODULATION IN THE BRAIN-GUT AXIS

Ashley Blackshaw and Terez Shea-Donohue, Presiding

8:00 ENTERIC NERVOUS SYSTEM: IMMUNE MODULATION AND PLASTICITY.
Jackie Wood, *The Ohio State University*

8:25 ROLE OF STAT4 IN THE REGULATION OF FUNCTIONAL AND IMMUNE RESPONSES IN INFECTIOUS COLITIS. *T Shea-Donohue*, AD Smith, RY Sun, JF Urban, and A Zhao, Baltimore and Beltsville, MD. *University of Maryland School of Medicine* ***(Abstract of Distinction)*** Abstract 16

8:40 POST-INFECTIOUS IRRITABLE BOWEL SYNDROME.
Stephen Collins, McMaster University

9:05 ON THE MECHANISMS OF ACQUIRED ENTERIC NEUROPATHIES: A MODEL OF HERPES SIMPLEX VIRUS-1 (HSV1) INFECTION OF THE RAT ENTERIC NERVOUS SYSTEM IN VIVO. *R De Giorgio*, P Brun, A Gori, V Stanghellini, G Barbara, C Felicani, G Palù, G Zaninotto, M Tonini, R Corinaldesi, and I Castagliuolo, Bologna, Padua, and Pavia, Italy. *Bologna University* ***(Abstract of Distinction)*** Abstract 17

9:20 IMMUNE, CYTOKINE AND CHEMOKINE MEDIATORS OF ILEUS.
Anthony Bauer, *University of Pittsburgh*

9:45 RECOVERY ROLE OF IL-10 IN POSTOPERATIVE ILEUS. *B Stoffels*, J Schmidt, A Mazie, S Pollard, and AJ Bauer, Pittsburgh, PA. *University of Pittsburgh* ***(Young Investigator Travel Awardee and Abstract of Distinction)*** Abstract 18

10:00 am – 10:15 am Intermission

NOVEL THERAPEUTIC MANAGEMENT OF GASTROINTESTINAL MOTILITY AND FUNCTIONAL GASTROINTESTINAL DISORDERS

10:15 AM – 12:15 PM
Harborview Ballroom

James Galligan and Yvette Taché, Presiding

10:15 PHARMACOGENOMICS: WHAT'S HOT, WHAT'S NOT?
Gerald Holtmann, *Royal Adelaide Hospital*

10:40 THE INFLUENCE OF CANDIDATE GENES ON THE RESPONSE OF SIBUTRAMINE TREATMENT FOR WEIGHT LOSS IN OVERWEIGHT AND OBESITY. *M Vazquez Roque*, M Camilleri, P Carlson, MM Clark, D Stephens, K Graszer, S Kalsy, and AR Zinsmeister,

Rochester, MN. *Mayo Clinic College of Medicine* ***(Young Investigator Travel Awardee and Abstract of Distinction)*** Abstract 19

10:55 SEROTONERGIC MECHANISMS IN FUNCTIONAL GI DISORDERS.
Michael Gershon, *Columbia University, College of Physicians and Surgeons*

11:20 5-HT$_4$ RECEPTOR-IMMUNOREACTIVITY (5-HT$_4$-IR) IS EXPRESSED IN NON-NEURONAL CELLS OF THE HUMAN GASTROINTESTINAL (GI) TRACT. *C Streutker*, EC Colley, K Hillsley, G Hicks, S Kelly, and RH Stead, Toronto, Bowmanville, Ontario, Canada, East Hanover, NJ, and Dorval, Quebec, Canada *St. Michael's Hospital* ***(Abstract of Distinction)*** Abstract 20

11:35 PSYCHOLOGICAL AND ALTERNATIVE THERAPEUTICS.
Douglas Drossman, *University of North Carolina*

12:00 VISCERAL AND GENERAL MEDICAL ANXIETY AS PREDICTORS OF IBS SYMPTOM SEVERITY. *MP Frese*, JS Labus, R Bolus, L Chang, E Mayer, and BD Naliboff, Los Angeles, CA. *David Geffen School of Medicine at UCLA* ***(Young Investigator Workshop Awardee and Abstract of Distinction)*** Abstract 21

LUNCH AND POSTER SESSION
12:15 PM – 3:00 PM
Seaport World Trade Center Exhibition Hall

CONCURRENT SESSIONS

NOVEL SIGNALING PATHWAYS

One of Three Concurrent Sessions
3:00 PM – 5:00 PM
Harborview Ballroom

Gianrico Farrugia and Michael Schemann, Presiding

3:00 NEUROEPITHELIUM AXIS.
Michel Neunlist, *INSERM*

3:25 ENTERIC GLIAL CELLS (EGC) SUPPORT THE INTESTINAL EPITHELIAL BARRIER. *S Hoff*, C Hank, M Schemann, and A Rühl, Munich and Freising, Germany. *Technical University Munich* ***(Young Investigator Travel Awardee and Abstract of Distinction)*** Abstract 22

3:40 NEURO-ICC AXIS.
Sean Ward, *University Nevada*

4:05 INCREASED PROLIFERATION OF INTERSTITIAL CELLS OF CAJAL DUE TO ALTERED DESENSITIZATION OF c-KIT. *AN Holm*, JL Roeder, MS Lurken, MM Wouters, N Borg, P Blume-Jensen, T Hunter, SJ Gibbons, and G Farrugia, Rochester, MN, Boston, MA, and LaJolla, CA. *Mayo Clinic College of Medicine* ***(Abstract of Distinction)*** Abstract 23

4:20 AGING AND GI SMOOTH MUSCLE: WHAT'S NEW AND WHAT'S POTENTIALLY REVERSIBLE?
Khalil N. Bitar, *University of Michigan*

4:45 H_2S MEDIATES SMOOTH MUSCLE RELAXATION THROUGH K CHANNELS IN HUMAN AND RAT COLON. *D Gallego*, M Beyak, P Clave, D Grundy, and M Jimenez, Barcelona, Spain, Kingston, Canada, and Sheffield, United Kingdom. *Universitat Autonoma de Barcelona* ***(Young Investigator Travel Awardee and Abstract of Distinction)*** Abstract 24

6:30 BANQUET AT HARVARD CLUB OF BOSTON – Meet in Seaport hotel lobby at 6:15 with banquet ticket.

NOVEL APPROACHES TO DIAGNOSIS

One of Three Concurrent Sessions
3:00 PM – 5:00 PM
Amphitheater

Lin Chang and Brad Kuo, Presiding

3:00 SYMPTOM DRIVEN DIAGNOSES.
Yuri Sato, *Mayo Clinic College of Medicine*

3:25 ROME II VS. ROME III CRITERIA FOR FUNCTIONAL GASTROINTESTINAL DISORDERS IN PEDIATRIC PATIENTS EVALUATED FOR ABDOMINAL PAIN.
KE Freeman, J Anderson, M Puzanovova, and LS Walker, Nashville, TN. *Vanderbilt University* ***(Abstract of Distinction)*** Abstract 25

3:40 IMAGE APPROACHES TO DIAGNOSES.
Juan Malagelada, *Hospital Universitari Vall d'Hebron*

4:05 CHARACTERIZATION OF THE PYLORIC SPHINCTER WITH HIGH RESOLUTION MANOMETRY. *FK Friedenberg*, J DeSipio, A Korimilli, and HP Parkman, Philadelphia, PA. *Temple University School of Medicine* ***(Abstract of Distinction)*** Abstract 26

4:20 HISTOPATHOLOGY IN DISORDERS OF GASTROINTESTINAL MOTILITY.
Roberto Degiorgio, *University of Bologna*

4:45 ENHANCED EXPRESSION OF GENES ASSOCIATED WITH VISCERAL HYPERSENSITIVITY IN SMALL INTESTINE OF IRRITABLE BOWEL SYNDROME PATIENTS. *JJ ter Linde*, AP Kerckhoffs, LM Akkermans, and M Samsom, Utrecht, The Netherlands. *University Medical Centre Utrecht* ***(Young Investigator Travel Awardee and Abstract of Distinction)*** Abstract 27

6:30 BANQUET AT HARVARD CLUB OF BOSTON – Meet in Seaport hotel lobby at 6:15 with banquet ticket.

CONCURRENT SESSIONS

METHODOLOGIES FOR HEALTH CARE RESEARCH

One of Three Concurrent Sessions
3:00 PM – 5:00 PM
Waterfront Ballroom

G. Richard Locke and Brennan Spiegel, Presiding

3:00 **EPIDEMIOLOGY AND DATA COLLECTION.**
E. Jan Irvine, *St. Michael's Hospital*

3:25 **IS HYPERSENSITIVITY TO DRUGS AN EXTRA GI SOMATIC MANIFESTATION OF IBS?** *A Gougeon*, P Poitras, M Binn, and M Bouin, Montreal, Quebec, Canada. *Hôpital Saint-Luc* ***(Abstract of Distinction)*** Abstract 28

3:40 **MEASUREMENT OF QUALITY OF LIFE AND IMPACT OF THERAPY.**
Brennan M.R. Spiegel, *David Geffen School of Medicine at UCLA*

4:05 **EXACERBATION OF IRRITABLE BOWEL SYNDROME SYMPTOMS DURING MENSES IS ASSOCIATED WITH INCREASED PROSTAGLANDIN (PGE_2) LEVELS.** *S Heymen*, M van Tilburg, S Thiwan, O Palsson, SL Young, and WE Whitehead, Chapel Hill, NC. *University of North Carolina - Chapel Hill* Abstract 29

4:20 **OUTCOME ASSESSMENT IN CLINICAL TRIALS.**
Sander J.O. Veldhuyzen VanZanten, *Dalhousie University*

R. Ueno presenting

4:45 **LONG-TERM SAFETY AND EFFICACY OF LUBIPROSTONE FOR THE TREATMENT OF CHRONIC IDIOPATHIC CONSTIPATION.** *JF Johanson*, R Panas, PC Holland, and R Ueno, Stamford, CT and Bethesda, MD. *Rockford Gastroenterology Associates* ***(Abstract of Distinction)*** Abstract 30

6:30 **BANQUET AT HARVARD CLUB OF BOSTON – Meet in Seaport hotel lobby at 6:15 with banquet ticket.**

PLENARY SESSSION

SUNDAY, SEPTEMBER 17, 2006
8:00 AM – 12:00 PM
Harborview Ballroom

REGULATION OF APPETITE AND OBESITY: WHERE ARE WE NOW AND WHERE ARE WE HEADED?

Frank Hamilton and John Wiley, Presiding

8:00 *Keynote Speaker*
MODELS OF METABOLISM: OF MICE AND MEN.
Charles Burant, *University of Michigan Health System*

9:00 **PERIPHERAL MECHANISMS IN THE REGULATION OF APPETITE AND OBESITY.**
James A. Levine, *Mayo Clinic College of Medicine*

9:25 **CENTRAL MECHANISMS IN THE REGULATION OF ENERGY AND APPETITE.**
Gary J. Schwartz, *Albert Einstein College of Medicine*

10:20 **APPETITE AND OBESITY: THE GASTROENTEROLOGIST'S PERSPECTIVE.**
Michael Camilleri, *Mayo Clinic College of Medicine*

10:45 *Keynote Speaker*
MODELING COMPLEX BIOLOGICAL SYSTEMS: WHERE ARE WE HEADED?
Leroy Hood, *Institute for Systems Biology*

11:45 **CONCLUDING REMARKS**
Henry Parkman and Michael Fried

LUNCH AND POSTER SESSION

FRIDAY, SEPTEMBER 15 AND SATURDAY, SEPTEMBER 16
12:15 PM – 3:00 PM
Exhibition Hall – World Trade Center

Anorectal Disorders

1 **ALTERNATIVE ANTIADRENERGIC PHARMACODYNAMICS OF BoNT/A.** *A Balletta*, M Runfola, M Di Mugno, and D Gui, Rome, Italy. *Catholic University* ***(Abstract of Distinction)*** Abstract 31

2 **LATE ONSET OF HIRSCHPRUNG`S DISEASE: A DIAGNOSTIC DILEMMA.** *M Bashashati*, MS Fazeli, M Hajirostam, MK Nouri-taromlu, and B Haghpanah, Tehran, Iran *Tehran University of Medical Science* Abstract 32

3 **MAGNETIC RESONANCE IMAGING OF IDIOPATHIC MEGARECTUM DURING DISTENSION.** *G Basilisco*, L Di Palma, C Tomba, and LV Forzenigo, Milan, Italy. *University of Milan* Abstract 33

4 **BIOFEEDBACK TREATMENT IMPROVES QUALITY OF LIFE IN PATIENTS WITH FECAL INCONTINENCE.** *MM Bosca*, M Minguez, MM Bosca, V Sanchiz, A Basagoiti, P Almela, F Mora, and A Benages, Valencia, Spain. *University of Valencia* Abstract 34

5 **THE FREQUENCY OF THE SAMPLING RESPONSE DOES NOT DETERMINE URGE TO DEFECATE IN PATIENTS WITH CONSTIPATION: A STUDY USING SEMI-AMBULATORY ANORECTAL PHYSIOLOGY.** *S Cowlam*, Y Yiannakou, D Wooff, and P Saunders, Durham, United Kingdom. *University Hospital North Durham* Abstract 35

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47 **HYDROGEN PEROXIDE CONTRIBUTES TO THE IMPAIRMENT OF CALCIUM RELEASE IN COLONIC SMOOTH MUSCLE CELLS IN COLITIS.** *W Cao* and VE Pricolo, Providence, RI. *Brown Medical School* Abstract 77

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66 **LUMINALLY RELEASED SEROTONIN STIMULATES COLONIC MOTILITY AND ACCELERATES COLONIC TRANSIT IN RATS.** *K Tsukamoto*, H Ariga, TN Pappas, T Takahashi, and C Mantyh, Durham, NC. *Duke University* ***(Abstract of Distinction)*** Abstract 96

67 **ABNORMALITIES OF THE ENTERIC NERVOUS SYSTEM, SMOOTH MUSCLE AND INTERSTITIAL CELLS OF CAJAL IN CHILDREN WITH COLONIC MOTILITY DISORDERS.** *MM van den Berg*, HM Mousa, C Di Lorenzo, MA Benninga, GE Boeckxstaens, and M Luquette, Amsterdam, The Netherlands and Columbus, OH. *Emma Children's Hospital / Academic Medical Center* ***(Young Investigator Workshop Awardee)*** Abstract 97

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81 **BETA-3 ADRENOCEPTOR AGONISM INHIBITS EXCITABILITY IN HUMAN ENTERIC NEURONS.** *N Hafsi*, J Wollmann, F Zeller, CW Hann, V. Weyern, R Thangiah, O Lalude, S Vivekanandan, GW Sanger, WJ Winchester, K Lee, S Cellek, and M Schemann, Freising, Munich, and Harlow, Germany. *Technical University Munich* ***(Young Investigator Travel Awardee)*** Abstract 111

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85 **MECHANISMS OF SYNAPTIC FACILITATION WITHIN THE MYENTERIC PLEXUS OF THE INFLAMED GUINEA PIG DISTAL COLON.** *EM Krauter*, DR Linden, KA Sharkey, and GM Mawe, Burlington, VT, Rochester, MN, and Calgary, Alberta, Canada. *University of Vermont* ***(Abstract of Distinction)*** Abstract 115

86 **MECHANISMS BEHIND THE SECRETORY RESPONSE TO DISTENSION IN DISTAL RAT DUODENUM IN VIVO.** *MH Larsson*, M Sapnara, JC Bornstein, H Sjövall, and E Lindström, Mölndal and Göteborg, Sweden, and Melbourne, Australia. *AstraZeneca R&D* Abstract 116

87 **HYDROGEN SULFIDE IS SYNTHESIZED IN THE MUSCLE LAYERS OF THE COLON AND IN PREVERTEBRAL GANGLIA AND ALTERS THE ELECTRICAL PROPERTIES OF NEURONS.** *DR Linden*, L Sha, A Mazzone, G Stoltz, C Bernard, JK Furne, MD Levitt, G Farrugia, and JH Szurszewski, Rochester and Minneapolis, MN. *Mayo Clinic* ***(Abstract of Distinction)*** Abstract 117

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89 **GDNF MEDIATED ENTERIC NEURONAL SURVIVAL INVOLVES GSK3β AND TAU.** *S Mwangi*, M Anitha, H Fu, and S Srinivasan, Atlanta, GA. *Emory University* Abstract 119

90 **NEUROPROTECTIVE EFFECTS OF ENTERIC GLIAL CELLS IN A MODEL OF DOPAMINE INDUCED-NEUROTOXICITY.** *M Neunlist*, JP Galmiche, D Masson, P Aubert, J Chevalier, M Hubert, P Gomes, H Abdo, B Lardeux, and P Vanden Berghe, Nantes, France and Leuven, Belgium. *INSERM* Abstract 120

91 **DEVELOPMENTAL REGULATION OF THE NEUROCHEMICAL PHENOTYPE OF THE ENS: IMPLICATION OF L-TYPE CA^{2+} CHANNELS.** *M Neunlist*, J Chevalier, P Gomes, R Thinard, P Naveilhan, JP Galmiche, and P Vanden Berghe, Nantes, France and Leuven. *INSERM* Abstract 121

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94 **PERIPHERAL GLIA REGULATE EPITHELIAL PERMEABILITY AT MUCOSAL SURFACES VIA RELEASE OF S-NITROSOGLUTATHIONE.** *TC Savidge*, P Newman, C Pothoulakis, A Ruhl, M Neunlist, A Bourreille, R Hurst, and M Sofroniew, Galveston, TX, Boston, MA, Munich, Germany, Nantes, France, Bristol, United Kingdom, and Los Angeles, CA. *University of Texas Medical Branch* Abstract 124

95 **SUBSTANCE P AND VASOACTIVE INTESTINAL PEPTIDE ARE LOW IN RIGHT COLON IN CHILDREN WITH SLOW TRANSIT CONSTIPATION.** *BR Southwell*, SK King, JR Sutcliffe, SY Ong, M Lee, PJ Farmer, P Hengel, MR Stanton, J Keck, CW Chow, JM

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98 PHARMACOLOGICAL SEPARATION OF THE GASTRO-ESOPHAGEAL SEGMENT INTO THREE DISTINCT SPHINCTERIC COMPONENTS. *JG Brasseur*, R Ulerich, Q Dai, D Patel, A Soliman, and LS Miller, University Park and Philadelphia, PA. *Pennsylvania State University* Abstract 128

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100 DISTINGUISHING GERD: SLIGHT DISTENSION OF THE GASTRO-ESOPHAGEAL SEGMENT GREATLY ENHANCES THE PROBABILITY FOR REFLUX, BUT COMPLIANCE DOES NOT. *JG Brasseur* and SK Ghosh, University Park, PA and Chicago, IL. *Pennsylvania State University* Abstract 130

101 AN ENDOSCOPIC IMPLANTABLE DEVICE STIMULATES THE LOWER ESOPHAGEAL SPHINCTER ON-DEMAND BY REMOTE CONTROL IN A CANINE MODEL. *JO Clarke*, SB Jagannath, DM Beitler, AN Kalloo, VR Long, and SV Kantsevoy, Baltimore, MD. *Johns Hopkins University* ***(Young Investigator Workshop Awardee)*** Abstract 131

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105 HEALTH-RELATED QUALITY OF LIFE AND SYMPTOM SCORES IN CLINICALLY ADEQUATELY TREATED ACHALASIA PATIENTS: A CROSS-SECTIONAL STUDY. *R Frankhuisen*, HG Gooszen, JR Vermeijden, A Baron, AJ Smout, R Heijkoop, MA van Herwaarden, and M Samsom, Utrecht and Amersfoort, The Netherlands. *University Medical Center Utrecht* Abstract 135

106 RELATIONSHIP BETWEEN UPPER GASTROINTESTINAL SYMPTOMS AND POSITIVE 24 HR ESOPHAGEAL PH TESTS IN THAI PATIENTS WITH CHRONIC UPPER GASTROINTESTINAL SYMPTOMS. *S Gonlachanvit* and P Samdin, Bangkok, Thailand. *Chulalongkorn University* Abstract 136

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108 DIAGNOSIS OF NON-ACID GASTROESOPHAGEAL REFLUX (GER) USING AMBULATORY MULTICHANNEL INTRALUMINAL IMPEDANCE AND pH (MIIpH) MEASUREMENT. *M Hirata*, DF Evans, and E Yazaki, London, United Kingdom. *Barts and The London School of Medicine and Dentistry* Abstract 138

109 IMPAIRED GASTRIC MOTILITY AND ITS RELATIONSHIP TO REFLUX SYMPTOMS IN PATIENTS WITH NONEROSIVE GASTROESOPHAGEAL REFLUX DISEASE. *T Kamiya*, H Adachi, M Hirako, M Shikano, E Matsuhisa, N Misu, and Y Kobayashi, Nagoya, Japan. *Nagoya City University Graduate School of Medical Sciences* Abstract 139

110 CLINICAL UTILITY OF ESOPHAGEAL MANOMETRY. *BE Lacy*, L Paquette, D Robertson, J Weiss, and ML Kelley, Jr, Lebanon, NH, White River Junction, VT, and Hanover, NH. *Dartmouth-Hitchcock Medical Center* Abstract 140

111 THE ROLE OF $GABA_A$ RECEPTORS IN THE CONTROL OF TRANSIENT LOWER ESOPHAGEAL SPHINCTER RELAXATIONS (TLESRS) IN THE DOG. *A Lehmann*, S Pierrou, A Jönsson-Rylander, J Jensen, L Brändén, H Beaumont, and GE Boeckxstaens, Mölndal, Sweden, and Amsterdam, The Netherlands. *AstraZeneca R&D Mölndal* Abstract 141

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113 ENDOSCOPIC PLICATION PARTIALLY RESTORES A MISSING TONIC PRESSURE COMPONENT TO THE LES AND THE STIFFNESS OF RELAXED ESOPHAGEAL MUSCLE DURING TLESR IN PATIENTS WITH GERD. *LS Miller*, Q Dai, BJ Schiffner, J Dimitriou, and JG Brasseur, University Park and Philadelphia, PA, and Cranston, RI. *Pennsylvania State University* Abstract 143

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117 CAN ESOPHAGEAL IMPEDANCE PREDICT THE OCCURRENCE OF POST-FUNDOPLICATION DYSPHAGIA? *NQ Nguyen*, K Ching, J Myers, M Tippett, GG Jamieson, and RH Holloway, Adelaide, Australia. *Royal Adelaide Hospital* Abstract 147

118 MORPHOMETRIC EVALUATION OF MUSCLE AND NEURONAL INNERVATION OF ESOPHAGEAL WALL IN PATIENTS WITH NUTCRACKER ESOPHAGUS AND INEFFECTIVE ESOPHAGEAL MOTILITY. *H Park*, JH Lim, and HS Kim, Seoul, Korea. *Yonsei University* Abstract 148

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120 MR FLUOROSCOPY IN THE ASSESSMENT OF ESOPHAGEAL MOTILITY DISORDERS. *L Piretta*, E Corazziari, V Panebianco, Fi Habib, D Badiali, M Anzidei, F Alghisi, and E Tomei, Rome, Italy. *Università La Sapienza* Abstract 150

121 MICROSTRUCTURAL VOLUMETRIC IMAGING OF A HUMAN GASTROESOPHAGEAL JUNCTION. *AJ Pullan*, R Yassi, LK Cheng, D Gerneke, I LeGrice, JA Windsor, and AJ Pullan, Auckland, New Zealand. *The University of Auckland* Abstract 151

122 ADENOSINE MEDIATES ESOPHAGEAL SENSORIMOTOR FUNCTION IN HEALTHY HUMANS. *JM Remes-Troche*, P Chahal, B Hayek, and SSC Rao, Iowa City. IA. *University of Iowa* ***(Young Investigator Workshop Awardee and Abstract of Distinction)*** Abstract 152

123 ESOPHAGEAL BARSOSTAT OR IMPEDANCE PLANIMETRY: WHICH IS BEST SUITED FOR ESOPHAGEAL SENSORY TESTING. *JM Remes-Troche*, P Chahal, B Hayek, and SSC Rao, Iowa City, IA. *University of Iowa* Abstract 153

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126 PROTON PUMP INHIBITOR (PPI) INFLUENCE ON REFLUX CLEARANCE IN BARRETT'S ESOPHAGUS. *A Smythe*, GP Troy, NC Bird, R Ackroyd, and AG Johnson, Sheffield, England. *Royal Hallamshire Hospital* Abstract 156

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129 **DISTENSION DURING GASTROESOPHAGEAL REFLUX: EFFECTS OF ACID INHIBITION AND CORRELATION WITH SYMPTOM.** *NA Tipnis*, RK Mittal, and R Rhee, Milwaukee, WI and San Diego, CA. *Medical College of Wisconsin* Abstract 159

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134 **METABOTROPIC GLUTAMATE 5 RECEPTORS CONTRIBUTE TO COLORECTAL MECHANICAL SENSITIVITY IN RATS.** *LA Blackshaw*, BD Phillis, M Brusberg, S Arvidsson, R Eriksson, V Martinez, H Larsson, LA Blackshaw, and E Lindstrom, Adelaide, Australila and Mölndal, Sweden. *Royal Adelaide Hospital* ***(Abstract of Distinction)*** Abstract 164

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136 **EXPERIMENTAL COLITIS IN RATS IMPAIRS GASTRIC EMPTYING VIA THE PELVIC NERVE.** *HU De Schepper*, JG De Man, L Van Nassauw, JP Timmermans, PA Pelckmans, and BY De Winter, Antwerp, Belgium. *University of Antwerp* ***(Young Investigator Workshop Awardee)*** Abstract 166

137 **ROLE OF AFFERENT NEURONS AND TRPV-1 RECEPTORS IN THE PATHOGENESIS OF SEPTIC ILEUS IN MICE.** *BY De Winter*, HU De Schepper, JG De Man, AJ Bredenoord, AG Herman, and PA Pelckmans, Antwerp, Belgium. *University of Antwerp* ***(Abstract of Distinction)*** Abstract 167

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140 NIPPOSTRONGYLUS BRASILIENSIS (NB)-INFECTED MICE EXHIBIT CHEMICAL BUT NOT MECHANICAL HYPERSENSITIVITY. *K Hillsley*, C McCaul, PJ Peeters, J Aerssens, D Grundy, B Coulie, and RH Stead, Bowmanville, Ontario, Canada, Beerse, Belgium, and Sheffield, United Kingdom. *St. Michael's Hospital* ***(Abstract of Distinction)*** Abstract 170

141 EXTRINSIC AFFERENTS SUPPLYING THE MURINE JEJUNUM EXPRESS FUNCTIONAL TRPA1 RECEPTORS. *K Hillsley*, J Lin, P Peeters, J Aerssens, D Grundy, B Coulie, and RH Stead, Bowmanville, Ontario, Canada, Beerse, Belgium, and Sheffield, United Kingdom. *St. Michael's Hospital* Abstract 171

142 MAJOR DEFICITS IN VISCERAL MECHANOSENSORY FUNCTION IN MICE LACKING THE TRPV4 ION CHANNEL. *PA Hughes*, SM Brierley, AJ Page, W Liedtke, and LA Blackshaw, Adelaide, Australia and Durham, NC. *Royal Adelaide Hospital* ***(Young Investigator Workshop Awardee and Abstract of Distinction)*** Abstract 172

143 GASTRIC DISTENSION-INDUCED BLOOD PRESSURE CHANGES IN CONSCIOUS RATS. *P Janssen*, M Astin Nielsen, PG Gillberg, and L Hultin, Mölndal, Sweden. *AstraZeneca R&D* Abstract 173

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147 CHARACTERIZATION OF T9-T10 SPINAL NEURONS WITH DUODENAL INPUT AND MODULATION BY GASTRIC ELECTRICAL STIMULATION IN RATS. *C Qin*, JZ Chen, J Zhang, and RD Foreman, Oklahoma City, OK. *University of Oklahoma* Abstract 177

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149 MECHANISMS OF DIETARY FREE GLUTAMATE SENSING BY THE RAT GASTRIC VAGUS. *H Uneyama*, A San Gabriel, T Tanaka, A Niijima, and K Torii, Kawasaki and Niigata, Japan. *Institute of Life Sciences, Ajinomoto Co., Inc.,* Abstract 179

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154 **RELATIONSHIP BETWEEN ABDOMINAL PAIN AND BOWEL FREQUENCY IN FUNCTIONAL CONSTIPATION.** *D Badiali*, G Bausano, P Magrini, and F Anzini, Rome, Italy. *Università La Sapienza* Abstract 184

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156 **THE DIAGNOSTIC VALUE OF ROME II CRITERIA IN IRRITABLE BOWEL SYNDROME: A PRELIMINARY STUDY ON IRANIAN PATIENTS.** *M Bashashati*, NE Daryani, and K Ghannadi, Tehran, Iran. *Imam Khomeini Hospital* Abstract 186

157 **SENSITIVITY MEASUREMENT AND SUBGROUPING OF SUBJECTS USING ELECTRONIC ANALOGUE SCALE.** *A Bayati*, P Jerndal, M Karpefors, and LM Akkermans, Mölndal, Sweden. *AstraZeneca R&D* Abstract 187

158 **IMPAIRED ACCOMMODATION IN THE RECTUM. A POSSIBLE PATHOPHYSIOLOGICAL MECHANISM FOR IRRITABLE BOWEL SYNDROME (IBS).** *A Bayati*, M Karpefors, P Jerndal, and LM Akkermans, Mölndal, Sweden. *AstraZeneca R&D* Abstract 188

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160 **PSEUDOAFFECTIVE RESPONSE TO COLORECTAL DISTENSION IN MICE: IS IT A PHYSIOLOGIC INDICATOR OF VISCERAL PERCEPTION?** *P Bercik*, N Al-Mutawaly, W Jackson, P Blennerhassett, and SM Collins, Hamilton, Ontario, Canada. *McMaster University* Abstract 190

161 **DECREASE OF POSTPRANDIAL SERUM MOTYLIN LEVEL MAY CONTRIBUTE TO DYSPEPTIC SYMPTOMS DEVELOPMENT IN ESRD PATIENTS.** *U Blaut*, M Stojakowska, PJ Thor, O Smolenski, I Rogatko, and K Sztefko, Cracow, Poland. *Collegium Medicum Jagiellonian University* Abstract 191

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163 **INCREASED CYTOKINE PRODUCTION BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN IBS IS ASSOCIATED WITH SOMATISATION.** *EB Campbell*, M Richards, S Foley, M Hastings, P Whorwell, Y Mahida, I Hall, K Neal, and R Spiller, Nottingham and Manchester, United Kingdom. *University of Nottingham* Abstract 193

164 **MAST CELLS IN HUMAN, RAT AND MOUSE GUT ARE 5-HT$_4$ RECEPTOR-IMMUNOREACTIVE (IR).** *EC Colley*, K Hillsley, C Streutker, G Hicks, S Kelly, and RH Stead, Bowmanville, Ontario, Toronto, Canada, East Hanover, NJ, and Dorval, Quebec, Canada. *St. Michael's Hospital* ***(Abstract of Distinction)*** Abstract 194

165 **PATIENTS WITH CLINICALLY DIFFERENT SYMPTOM-BASED DIAGNOSIS OF FUNCTIONAL BOWEL DISORDERS MAY HAVE IDENTICAL SPECIFIC AND NON SPECIFIC ILEO-COLO-RECTAL MICROSCOPIC ABNORMALITIES.** *E Corazziari*, A Marcheggiano, A Covotta, C Iannoni, D Badiali, R Cantarini, M Paoletti, and N Pallotta, Rome, Italy. *Università La Sapienza* Abstract 195

166 **SLEEP DISTURBANCE IS AN INDEPENDENT PREDICTOR OF CHRONIC UPPER AND LOWER GASTROINTESTINAL (GI) SYMPTOMS: A POPULATION-BASED STUDY.** *F Cremonini*, GR Locke, AR Zinsmeister, M Camilleri, and NJ Talley, Rochester, MN. *Mayo Clinic and Foundation* Abstract 196

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167 **A META-ANALYSIS OF THE PLACEBO RESPONSE IN COMPLEMENTARY AND ALTERNATIVE MEDICINE TRIALS OF IRRITABLE BOWEL SYNDROME.** *SD Dorn*, AJ Lembo, J Park, K Canenguez, BH Nam, LA Conboy, WB Stason, and TJ Kaptchuk, Chapel Hill, NC and Boston, MA. *University of North Carolina* Abstract 197

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169 **WHAT GENERAL PRACTIONERS KNOW ABOUT IBS IN ROMANIA.** *DL Dumitrascu*, G Zagrean, and L David, Cluj, Romania. *Iuliu Hatieganu Cluj* Abstract 199

170 **RAPID EARLY PHASE GASTRIC EMPTYING MAY BE A CHARACTERISTIC OF CYCLIC VOMITING SYNDROME: A COMPARISON WITH FUNCTIONAL VOMITING.** *NR Fajardo* and GR Locke, III, Rochester, MN. *Mayo Clinic College of Medicine* Abstract 200

171 **MYOELECTRIC AND AUTONOMIC NERVOUS SYSTEM ACTIVITY IN PATIENTS WITH IRRITABLE BOWEL SYNDROME.** *A Furgala*, M Mazur, K Jablonski, D Madroszkiewicz, I Ciecko-Michalska, and PJ Thor, Cracow, Poland. *Jagiellonian University, Medical College* Abstract 201

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173 **VAGAL BIOFEEDBACK AND BREATHING EXERCISES: PROMISING TREATMENT OF FUNCTIONAL DYSPEPSIA?** *T Hausken*, A Berstad, S Svebak, and G Flatabø, Bergen, Trondheim, Ulvik, Norway. *University of Bergen* ***(Abstract of Distinction)*** Abstract 203

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175 **INCREASED PLASMA CATECHOLAMINES IN WOMEN WITH CONSTIPATION-PREDOMINANT IRRITABLE BOWEL SYNDROME DURING SLEEP.** *MM Heitkemper*, M Jarrett, R Burr, K Cain, and A Poppe, Seattle, WA. *University of Washington* Abstract 205

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178 **RECTAL MOTOR PHYSIOLOGY IN DIARRHEA-PREDOMINANT IRRITABLE BOWEL SYNDROME AND INFLUENCE OF ACUTE SEROTONERGIC MODULATION.** *T Kilkens*, MV Nieuwenhoven, and RJ Brummer, Maastricht, The Netherlands. *Maastricht University* Abstract 208

179 **VAGAL AFFERENTS DISCHARGE AND MIOELECTRICAL ACTIVITY IN GASTRIC HYPERALGESIA IN RATS.** *G Krolczyk*, K Gil, D Zurowski, A Jung, J Sobocki, and PJ Thor, Cracow, Poland. *Jagiellonian University* Abstract 209

180 **PREVALENCE OF FUNCTIONAL GASTROINTESTINAL DISORDERS AND RELEVANCE OF ANXIETY AND DEPRESSION TO FUNCTIONAL GASTROINTESTINAL DISORDERS: A POPULATION-BASED STUDY IN SOUTH KOREA.** *KJ Lee*, SY Lee, SJ Kim, SJ Shin, KB Hahm, JH Kim, and SW Cho, Suwon, South Korea. *Ajou University School of Medicine* Abstract 210

181 **A 6 MONTH PROGNOSTIC MODEL FOR IBS WITHOUT DIARRHEA IN PRIMARY CARE: THE IRRITABLE BOWEL SYNDROME LONGITUDINAL OUTCOMES STUDY (ILOS).** *GR Locke*, KH Kahler, N Lesnikova, and RF Balshaw, Rochester, MN, Vancouver, BC and E. Hanover, NJ. *Mayo Clinic College of Medicine* Abstract 211

182 **RESULTS OF GLUCOSE BREATH TESTING FOR SMALL INTESTINAL BACTERIAL OVERGROWTH (SIBO) IN IRRITABLE BOWEL SYNDROME (IBS) PATIENTS: CLINICAL PROFILES AND EFFECTS OF RIFAXIMIN TREATMENT.** *M Majewski*, S Sostarich, P Foran, and RW McCallum, Kansas City, KS. *The University of Kansas Medical Center* Abstract 212

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371 **ALTERED ELECTRICAL ACTIVITY IN DUODENAL MUSCLE CELLS FROM MDX (DYSTROPHIC) MICE.** *R Serio*, MG Zizzo, A Montalbano, and F Mulè, Palermo, Italy. *Università di Palermo* Abstract 401

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374 **α-SMA IMMUNOREACTIVITY IN THE NORMAL HUMAN ILEUM: PLAYING FOOL PLAY?** *JM Vanderwinden*, T Wedel, JP Bogers, and D Waltrégny, Brussels, Antwerpen, and Liège, Belgium and Kiel, Germany *Universite Libre de Bruxelles* Abstract 404

INVITED SPEAKER TALKS

(assembled in chronological order)

ENTERIC NERVES AND GLIAL CELLS IN HEALTH AND DISEASE

Simon Brookes, Ph.D.
Flinders University

The enteric nervous system contains millions of neurons including primary afferent (sensory) neurons, interneurons and motor neurons, arranged in several ganglionated plexuses. Discrete identifiable classes can be distinguished by their projections, combinations of neurochemicals, soma-dendritic morphology, electrophysiological features, combinations of receptors and their patterns of connectivity. In a few preparations, all of the classes of cells have been distinguished, revealing that there are fewer than 15-20 different types in any region of gut. The detailed combinations of characteristics shows significant variation between different regions of gut within one species and between the same region of gut in different species. Despite this, common features are numerous and a picture of how the enteric nervous system functions is gradually emerging.

Enteric neural circuits are activated via specific classes of neurons, which respond to substances released from mucosal cells and by some chemical stimuli. Several classes of enteric nerve cells respond to mechanical deformation to activate motor patterns; some of these classes also function as interneurons or even as effector cells. In vivo, enteric circuits are modulated by sympathetic, parasympathetic and extrinsic sensory neuron collaterals, allowing coordination between distant regions of gut and central modulation of gut function. In turn, viscerofugal enteric circuits modulate these extrinsic efferent pathways.

Enteric glia outnumber enteric neurons and are located in myenteric and submucous ganglia, in non-ganglionated plexuses and in the mucosa. Glial cells show electrical and dye coupling. While they play a role in trophic and mechanical support of enteric neurons, they can also respond to neurotransmitters and interact strongly with the immune system. A detailed picture of enteric neurons and glia has emerged from the last 30 years of research. A considerable challenge is to characterize the specific mechanisms which are altered in genetic and developmental disorders and in a wide range of disease states, and relate these to the generation of symptoms.

INTERSTITIAL CELLS OF CAJAL IN HEALTH AND DISEASE

Gianrico Farrugia, M.D.
Enteric NeuroScience Program
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Our understanding of the different roles interstitial cells of Cajal (ICC) play in the control of gastrointestinal motility has increased significantly in recent years. ICC generate electrical signals and propagate these signals to smooth muscle cells to set contractile frequency and regulate contractility, ICC hyperpolarize smooth muscle membrane potential and set the membrane potential gradient, ICC amplify neuronal signals and act as mechanotransducers. The mechanisms by which these functions are carried out are now beginning to be better understood. Concomitant with our increased understanding of ICC roles and function has been an increased understanding of the role ICC may play in the pathogenesis of motility disorders of the gut, with the best evidence published for gastroparesis and slow transit constipation. Loss of ICC appears to be a central feature of these diseases. ICC numbers are not static, rather ICC networks appear to be constantly turning over and the mechanisms involved are just beginning to be understood. In disease states it appears that, rather than a simple loss of ICC, the reduced numbers of ICC observed are due to an altered balance between proliferation and cell death, the latter though different mechanisms including transdifferentiation and apoptosis. The realization that ICC numbers are reduced in certain motility disorders has lead to many publications showing altered ICC in a large variety of motility disorders and the introduction of ICC stains in routine pathology. However, the fixation, visualization, and quantification methods have not been standardized making comparisons between studies and between controls and diseased tissue difficult. This presentation will discuss the function of ICC in the gastrointestinal tract and the role ICC play in the pathophysiology of motility disorders of the gut. The talk will also discuss standardization of the fixation, visualization and quantification of ICC using light microscopy for research and clinical practice.

SMOOTH MUSCLE IN HEALTH AND DISEASE

Karnam S. Murthy, Ph.D.
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Virginia Commonwealth University

The primary contractile function of the smooth muscle is defined by the expression of selective repertoire of genes (e.g., smooth muscle-specific actin and myosin heavy chain). Smooth muscle of the gut possesses distinct regional and functional properties that distinguish it from other types of visceral and vascular smooth muscle. Smooth muscle of the proximal stomach, sphincters, and gall bladder exhibits sustained tone, whereas smooth muscle of the distal stomach, small intestine and colon exhibits variable tone on which are superimposed rhythmic contractions driven by cycles of membrane depolarization and repolarization known as slow waves that originate in interstial cells of Cajal.

Excitatory transmitters released from the enteric motor neurons accentuate depolarization and Ca^{2+} mobilization, and induce muscle contraction. Phosphorylation of Ser^{19} on myosin light chains (MLC_{20}) by Ca^{2+}/calmodulin-dependent MLC kinase is essential for initiation of smooth muscle contraction. The initial $[Ca^{2+}]_i$ transient is rapidly dissipated and MLC kinase inactivated, while MLC_{20} and muscle contraction are sustained. The sustained contraction involves (i) RhoA activation, (ii) RhoA-dependent regulated inhibition of MLC phosphatase via phosphorylation of the regulatory subunit of MLC phosphatase, MYPT1, and/or PKC-mediated phosphorylation of CPI-17, an endogenous inhibitor of MLC phosphatase, and (iii) MLC_{20} phosphorylation via a Ca^{2+}-independent MLC kinase. Inhibitory neurotransmitters released from the enteric motor neurons induce hyperpolarization and muscle contraction. Relaxation of contracted smooth muscle is mediated by cAMP-dependent kinase (PKA) and cGMP-dependent kinase (PKG). PKA and PKG act on several targets in the cascade that mediate initial and sustained contraction, resulting in MLC_{20} dephosphorylation and relaxation. MLC_{20} phosphorylation and dephosphorylation are the essential prerequisites of smooth muscle contraction and relaxation, respectively, and are thus the crucial biochemical markers of each.

Alteration in smooth muscle function occurs in variety of diseases such as inflammatory bowel disease. Smooth muscle cells act as both source and target of pro- and anti-inflammatory mediators setting up a dynamic balance that maintains the inflammatory response initially and facilitates its eventual resolution. The effect of inflammatory mediators on smooth muscle function could results from changes in the expression and activity of various components in the signaling cascade that mediate contraction and relaxation.

VISCERAL PERCEPTION: AFFERENT PATHWAYS

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Director, Center for Pain Research
University of Pittsburgh

The principal undesirable conscious sensations that arise from the gastrointestinal (GI) tract are bloating, discomfort and pain, which are usually produced by mechanical stimulation (e.g., hollow organ distension). The vast majority of chemical and mechanical events within the normal GI tract, however, do not lead to conscious perception of the event. The extrinsic (afferent) innervation of the GI tract has been well characterized:

- the axons are thinly myelinated (Aδ fibers) and unmyelinated (C fibers)
- their cell bodies are mostly medium-sized in diameter
- the axons traverse pre- and paravertebral ganglia en route to the spinal cord
- the number of afferents are few, but they extensively arborize in spinal cord
- visceral afferents terminate in spinal cord lamina I, IIo, V and X
- viscerosomatic and viscerovisceral convergence is common on central neurons
- visceral afferents are typically polymodal (chemo-, thermo- and/or mechanosensitive)

When the GI tract is experimentally insulted, visceral afferent neurons sensitize. That is, their response characteristics and thresholds for response change, and they become more easily excitable. Accordingly, previously innocuous stimuli which would not normally lead to conscious perception of GI events now generate greater input to the central nervous system and can lead to sensations of discomfort or pain.

Because the visceral afferent innervation exhibits considerable plasticity in response to intralumenal stimuli, it can be argued that most if not all visceral afferent fibers can contribute to the undesirable conscious sensations that arise from the GI tract. To examine the validity of this argument, it is important to more fully understand differences in selectivity of visceral receptors for relevant stimuli, differences between the functions of the two nerves that innervate each viscus, and the molecules that contribute to visceral chemo- and mechanosensitivity. These issues will be the focus of this presentation.

VISCERAL PERCEPTION: THE ROLE OF ENDOGENOUS MODULATION

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Chemical, mechanical and even some inflammatory stimuli of the gastrointestinal tract remain generally unperceived, despite ongoing activation of primary and secondary afferent neurons. In contrast to somatic, in particular cutaneous stimuli, the primary function of visceral afferents is not the mediation of conscious perception, but rather the triggering of homeostatic reflex responses along the entire brain gut axis. Under normal circumstances, the feeling of satiety and of rectal fullness are the only two situations when conscious perception is adaptive to trigger an appropriate behavioral response. But even under these circumstances, the brain is able to enhance or downregulate the afferent signal depending on the context, thoughts and feelings of the individual. Thus, there is no linear correlation between peripheral events within the GI tract and perception of GI sensations, suggesting an important role of the CNS in the downregulation (in the healthy individual) and upregulation (during stress, anxiety and in functional GI disorders) of visceral perception. Brainstem regions, including the periaqueductal grey (PAG) and the rostroventral medulla (RVM) play important roles in both descending inhibition and facilitation of afferent signals at the spinal cord level. Cortico-limbic inputs into these pontine pain modulation systems allow for cognitive and emotional modulation of visceral perception. Modulation occurs also at the cortical level itself, as evidenced by the release of endogenous opioids in various brain regions during sustained painful stimulation. Recent evidence from brain imaging studies in GI patient populations are consistent with an alteration of endogenous pain modulation systems, including ineffective activation of descending inhibition, and exaggerated activation of descending facilitation.

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VISCERAL PERCEPTION: COGNITIVE AND PSYCHOLOGICAL MECHANISMS

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Two-thirds of patients with irritable bowel syndrome (IBS) report abdominal discomfort or pain at a lower threshold than healthy controls. The question is whether these differences in verbal reports of subjective experience are due to biological differences, psychological and cognitive influences, or a combination. Evidence favoring a biological interpretation include (1) sensitization by peripheral inflammation, (2) secondary (central) sensitization, and (3) greater increases in cerebral blood flow in response to subliminal (weak, unperceived) distensions of the rectum. Evidence favoring a psychological/cognitive interpretation includes (1) manipulation of discomfort thresholds by attention and distraction, (2) influence of experimental stress on reports of pain intensity, (3) changes in cerebral blood flow in response to sham rectal distensions, and (4) correlations (modest but significant) between pain thresholds and psychological traits of anxiety and somatization. In two experiments we have used sensory decision theory (SDT) to distinguish between biological (neurosensory) factors and psychological (perceptual response bias) factors that might affect pain reporting. Neurosensory influences on perception are measured by the ability of subjects to distinguish between different intensities of painful distention. This ability is influenced by analgesia but not by psychological manipulations. Perceptual response bias, on the other hand, quantifies the tendency to call stimuli "painful" independent of their physical intensity, and this can be affected by altering the consequences (e.g. offering rewards) of reporting or not reporting pain. We confirmed that perception thresholds measured by the traditional method (ascending method of limits) are lower in IBS patients. These lower thresholds in IBS correlated with higher perceptual response bias and with psychological traits of anxiety and somatization; however, the ability to discriminate between painful stimuli was no different between IBS and controls and was not correlated with psychological traits. We conclude that pain sensitivity in IBS is determined more by psychological and cognitive than by biological factors.

INTERSTITIAL CELLS OF CAJAL: PHARMACOLOGY AND ROLE IN INTRINSIC PACING

Kenton Sanders, Ph.D.
University of Nevada

The smooth muscles of the gastrointestinal (GI) tract have intrinsic electrical rhythmicity (slow waves) that moves membrane potential between levels where there is very little Ca^{2+} entry to more depolarized potentials in which Ca^{2+} entry is enhanced and excitation-contraction coupling is accomplished. Thus, GI rhythmicity organizes electrical activity into a series of phasic contractions which are critical for the normal patterns of motility. Studies over the past decade have shown that interstitital cells of Cajal (ICC) provide the pacemaker activity that generates spontaneous electrical rhythmicity. This presentation will address the normal factors that regulate electrical pacing in GI muscles and the effects of pathophysiological factors on pacemaker frequency.

GASTRIC ELECTRICAL STIMULATION AND PACING: MECHANISMS, PARAMETERS, AND RESULTS

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University of Texas Medical Branch

Over the past 20 years, gastric electrical stimulation (GES) has received increasing attention among researchers and clinicians. Recently GES has been applied for treating gastroparesis as well as patients with obesity. The aim of this talk is to give a brief systematic review on the methodologies, results and mechanisms of GES. First, various methods of GES will be introduced and explained, including different stimulation locations and parameters. Secondly, the peripheral and central effects of GES will be reported. Peripherally, GES has been reported to alter gastric tone, gastric slow waves, antral contractions and gastric emptying. Both inhibitory and excitatory effects have been observed, depending on the methodologies of GES. Centrally, GES has been found to alter vagal afferent nerves, sympathetic afferent nerves, and central neuronal and humoral activities in various nuclei in the hypothalamus. Thirdly, potential applications of GES for treating gastroparesis as well as obesity will be discussed. A number of clinical studies have reported the suppression of nausea and vomiting in patients with gastroparesis. However, little is known about the possible mechanisms. On the opposite, GES has also been applied to treat patients with obesity. Clinical results and possible mechanisms regarding the application of GES for obesity will be presented. Finally, emerging technologies and future directions on GES will be introduced.

COLORECTAL ELECTRICAL STIMULATION: WHY, WHO AND HOW

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The treatment of faecal incontinence has traditionally focussed largely on the anal sphincter. Despite the advent of anal endosonography, which allowed accurate sphincter pathology to be characterised, the results of such treatment have often been poor. External anal sphincter trauma, including obstetric injury, has been treated by surgical repair. Degeneration or disruption of the internal anal sphincter is not amenable to surgical repair and has been treated by sphincter bulking injections. None of these therapies has been shown to be successful in the long term.

An alternative approach involves modifying gut and pelvic floor function using neurological manipulation. Sacral nerve stimulation (SNS) involves passing a fine wire electrode through a sacral foramen, most commonly S3, and chronically stimulating at a low frequency using an implanted battery stimulator.

SNS has been shown to provide long-term improved continence. It has been used to treat patients with internal sphincter weakness (structurally intact or not), external sphincter disruption, and incontinence after rectal prolapse surgery, partial spinal cord injury, and anterior resection for cancer.

SNS has been used to treat patients with intractable constipation who have failed conservative therapies. The benefit appears to be maintained at least in the medium term. A multicentre study is currently nearing completion.

Low level chronic stimulation affects sensory, motor and autonomic function. Rectal contractile activity may be diminished, and anal slow wave activity enhanced. Double blind studies have demonstrated that the benefit is not placebo mediated. SNS can be tested for three weeks using temporary stimulation; permanent implantation is considered only if the patient responds.

The amplitude of stimulation with SNS is much lower than the high amplitude short stimulation employed by the Brindley stimulator to treat spinally injured patients; the latter device produces large bowel peristalsis and sphincter relaxation, and can be used to facilitate bowel emptying.

Another form of electrical stimulation includes stimulation of the large bowel using directly implanted colonic electrodes. Such therapy has not been successful to date.

CANNABINOIDS AND THEIR RECEPTORS IN THE GI TRACT

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Preparations made from the plant *Cannabis sativa* have been used for centuries for the treatment of gastrointestinal (GI) tract disorders. Beneficial effects of treatment with cannabinoids (CB) include stimulation of appetite, inhibition of emesis, attenuation of abdominal pain and diarrhoea due to acute or chronic intestinal inflammation. To date we know that these actions are largely mediated by two G-protein coupled receptors, the CB_1 and CB_2 receptor. In the enteric nervous system, CB_1 receptors are located on enteric nerve terminals where they exert inhibitory actions on neurotransmission to reduce motility and secretion. Endogenous ligands for the CB receptors, termed endocannabinoids, can be rapidly synthesized from membrane lipids, and are present within the enteric nervous system, as are the degradative enzymes necessary to terminate their action. Interestingly, endocannabinoids not only act at cannabinoid receptors, but potentially also at vanilloid (TRPV1) and 5-HT_3 receptors, both of which are expressed throughout the gastrointestinal tract. The interactions between endocannabinoids and these other receptor systems are not fully understood.

For the regulation of motility, CB_1 receptors are localized presynaptically, where activation causes inhibition of neurotransmitter release. A physiologically active endocannabinoid tone results in the continued suppression of motility, which can be overcome by CB receptor antagonists. CB_1 and CB_2 receptors within the gut wall were reported to play an important role in the regulation of gastrointestinal inflammatory states. The expression and activity of these receptors is altered during inflammation, which may in turn also be regulated by activation of these receptors. The balance between CB_1 and CB_2 receptors may be important in pathophysiological states. The finding that the accelerated gastrointestinal transit observed in models of inflammation was shown to be reduced to control values by CB_2 but not CB_1 receptor agonists, which in normal animals had no effects on transit, illustrates this idea. CB_1 and CB_2 receptors are also present in the brainstem centres that regulate GI function extrinsically. Here, pharmacological activation of these receptors inhibits emesis and enhanced endocannabinoid levels *in vivo* are reported to be anti-emetic.

A full understanding of the endocannabinoid system within the GI tract under physiological and pathophysiological conditions could lead to major advances with important therapeutic potential in the treatment of GI disorders involving disturbances of motility and sensation, as well as inflammatory bowel diseases.

PROTEASE-ACTIVATED RECEPTORS

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Protease activated receptors belong to a family of seven transmembrane domain G- protein-coupled receptors that are activated by cleavage of their N-terminal domain by a proteolytic enzyme. The unmasked new N-terminal sequence acts as a tethered ligand that binds and activates the receptor itself. PARs are expressed throughout the gastrointestinal tract on several cell types, as enterocytes, mast cells, smooth muscle cells, myenteric neurons and endothelial cells. Immunohistochemical study indicated that PAR2 are mainly localized on apical site of colonic epithelial cells. In vivo, intracolonic activation of PAR2 led to colonic inflammation in mice and increased paracellular permeability with bacterial translocation into peritoneal organs. In mice, intracolonic infusion of low dose PAR2activating peptide (SLIGRL) increases colonic paracellular permeability by a direct MLCK-dependent mechanism. PAR2 activation induced colitis is dependent on sensory neuron activation, substance P and CGRP release. Recent preliminary works demonstrate an increased trypsin-like proteolytic activity in colonic biopsies from IBS patients able to cause hyperalgesia when injected into mouse paws thoroughly a mechanism involving PAR2activation.

Proteases are present in great amount in the gastrointestinal tract. In addition to their digestive role in protein degradation, they play a role as signaling molecules regulating cell functions by cleaving PARs. PARs are activated by a variety of proteases, such as digestive enzymes (trypsin and trypsinogen), proteases released from mast cells and neutrophils. However, resident colonic bacteria also release considerable amount of proteases susceptible and it has been hypothesize that bacterial proteases or at least luminal proteases of the cited origins may also act on PARs on colonocytes to affect colonic paracellular permeability by modulating the degree of PAR2activation and to produce visceral hypersensitivity to mechanical stimuli.

Accordingly, it has been recently identified that fecal material from patients exhibiting diarrhea-predominant Irritable Bowel Syndrome (IBS-D) have a 3-4 higher serine protease activity than controls or patients with infectious diarrhea. Fecal supernatants from IBS-D patients infused intracolonically in mice initiate colorectal hypersensitivity and increase colonic paracellular permeability of colonic strips in vitro. All these data suggest that activation of PAR-2 receptors on apical site of colonocytes may play a role in the genesis of symptoms in IBS-D patients.

AUTOIMMUNE MECHANISMS IN AUTONOMIC NEUROPATHY

Vanda Lennon, M.D., Ph.D.
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Autoimmunity can selectively target neurons in ganglia of the enteric nervous system and in extrinsic autonomic ganglia. This is well documented clinically in a paraneoplastic context, typically with small-cell lung carcinoma or thymoma. We have recently recognized an idiopathic form of autoimmune GI dysmotility (AGID) that, at least in some cases, is reversible. AGID and other partial forms of autoimmune autonomic neuropathy are currently under-appreciated as a clinical entity, but collectively they are conceivably as common as autoimmune endocrine disorders. The only proven effector of AGID to date is IgG that specifically targets the extracellular domain of neuronal ganglionic nicotinic acetylcholine receptors (AChR). Rabbits immunized with recombinant fragments of the ganglionic AChR α3 subunit (residues 1-205) develop profound and long-lasting GI dysmotility. Its clinical severity correlates with antibody titer. Furthermore, mice develop transient (i.e., reversible) dysmotility after injection of ganglionic AChR-IgG prepared from serum of immunized rabbits or patients with AGID. We have demonstrated antigen-specific activation of T cells in the rabbit model, but have not found evidence of ganglionic inflammation in either model of AGID.

Other diagnostically useful serological markers of AGID include autoantibodies reactive with skeletal muscle AChR and striational antigens, neuronal voltage-gated calcium channels (P/Q-type and N-type) and potassium channels, and neuronal nuclei (e.g., ANNA-1) and cytoplasm (e.g., CRMP-5). ANNA-1 and CRMP-5 autoantibodies are largely restricted to paraneoplastic cases of AGID, which generally are distinguishable from idiopathic AGID by a highly inflammatory histopathology. Ganglionic tissues are infiltrated by cytotoxic T cells that are known to be activated concomitantly with B-cells destined to secrete neuronal nuclear and cytoplasmic autoantibodies. In addition to investigating the capacity of neuronal nuclear and cytoplasmic antigens to elicit enteric ganglionitis, further evaluation of autoantibody profiles in patients with AGID may reveal novel candidate antigens in enteric glial cells or interstitial cells of Cajal.

CONSTIPATION IN CHILDHOOD

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To date, the worldwide prevalence of symptoms of constipation in children varies widely and is estimated to range between 0.3 - 28%.[1] Childhood constipation is recognized by infrequent, hard stools and the involuntary loss of faeces in the underwear. It is a debilitating condition that is often associated with abdominal pain. It causes distress to the child and to the family and can result in severe emotional disturbance and family discord. Importantly, childhood constipation often continues into adulthood and it is suggested that longstanding faecal impaction from childhood-on may result in a more complex pathophysiological end-state later in life. Increased rectal compliance but not decreased rectal sensitivity is the major pathophysiological mechanism in children with chronic constipation. Because of this increased compliance, these children require larger stool volumes to reach the intrarectal pressure threshold that triggers the sensation of the urge to defecate.[2]

Currently acute simple constipation is usually treated with a high fibre diet and sufficient fluid intake, filling out a diary and toilet training. The treatment of chronic constipation consists of 4 important phases: 1) education, 2) disimpaction, 3) prevention of re-accumulation of faeces and 4) follow up.
A double-blind randomized controlled study in healthy infants comparing a fructo-oligosaccharide-supplemented cereal (0.75 g FOS per cereal) with placebo showed a significant increase in mean numbers of stool per infant and softer stools.[3] The FOS supplemented cereal was well tolerated. A new promising probiotic strain, the probiotic strain Bifidobacterium Animalis, shortened both the total colonic transit time as the rectosigmoid transit time in healthy women.[4] A recent randomized double-blind controlled trial in IBS patients with constipation (<3 bowel movements/week), showed a significant increase, as compared to control, in stool frequency over the 6-weeks BA consumption.[5]

Two large double blinded, randomised controlled trial has been carried out showing that PEG and lactulose both significantly increase defecation frequency and decrease fecal incontinence frequency in children with constipation.[6,7]

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CYCLIC VOMITING SYNDROME

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Cyclic vomiting syndrome (CVS) is a severe, recurring vomiting disorder of children and adults that is currently diagnosed by the consensus criteria of 1994 (children, median 5 years) and Rome III criteria of 2006 (adults, median 34 years). The distinguishing clinical symptoms include discrete, stereotypical episodes characterized by pallor, listlessness, intense vomiting often up to 4-6 emeses/hour with unremitting nausea, severe abdominal pain and dehydration lasting for hours to 10 days. Despite its episodic nature and return to normal health between, CVS induces remarkable physiologic and psychological suffering, loss of academic and work productivity, and high medical costs for sufferers ($17,000/year). Important co-morbidities include affective disorders including anxiety (panic anxiety) and depression. Although CVS remains idiopathic, there is a known migraine connection, and recently proposed mechanisms include episodic autonomic dysfunction, mitochondrial DNA mutations causing cellular energy deficits and/or heightened hypothalamic stress responses activating the emetic response.

Although the differential diagnosis extends from inside (peptic, allergic, obstructive, dysmotile) to outside the GI tract (endocrine, metabolic, renal, neurologic), the extent of recommended exclusionary laboratory, radiographic, endoscopic, motility remains controversial. New NASPGHAN pediatric guidelines will recommend limited screening (UGI x-ray, glucose and electrolytes) unless alarm symptoms are present: bilious vomiting, abdominal tenderness/pain, attacks precipitated by fasting, an abnormal neurologic exam or progressive worsening. Successful management is multifaceted. These include lifestyle changes (migraine-type), stress reduction, support from the CVS Association, and cessation of cannabis. Empiric pharmacologic approaches include prophylactic (antimigraine agents, especially tricyclic antidepressants) therapy, abortive (tripans or setrons) agents administered at the onset of the attack, and the combination use of antiemetics (setrons), analgesics (NSAIDs and narcotics) and sedatives (benzodiazepines, phenothiazines) during episodes. Providing a treatment protocol can facilitate management in the hospital setting.

CHILDREN WITH FUNCTIONAL GASTROINTESTINAL DISORDERS: SHORT-TERM AND LONG-TERM CLINICAL OUTCOMES

Lynn S. Walker, Ph.D.
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When children are diagnosed with a functional gastrointestinal disorder (FGID), one of parents' first questions is, "Will my child get better?" Until recently, retrospective studies were the only empirical source of information to answer this question. These studies generally estimated that approximately 50% of youth with persistent abdominal pain would continue to complain of abdominal pain several years following a medical evaluation that yielded no evidence of organic disease. No data were available to assist the practitioner in identifying which children were likely to recover and which children were likely to maintain their symptoms. Recently, prospective studies of well characterized patients have begun to identify predictors of short and long-term outcomes of pediatric FGIDs. Results of those studies provide information on the prognostic utility of several factors including pain duration, pain severity, non-GI somatic symptoms, disability, psychosocial adjustment, life stress, and parent behavior. A developmental biopsychosocial model integrates these findings and suggests potential mechanisms that may contribute to the course and outcomes of pediatric FGIDs. This model can be used to guide future clinical outcome research and to inform clinician judgments regarding the prognosis for individual patients with FGIDs.

ENTERIC NERVOUS SYSTEM: IMMUNEMODULATION AND PLASTICITY

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Minute-to-minute gastrointestinal behavior, whether normal or disordered, is determined by integrative functions of the enteric nervous system (ENS). Input signals processed by the ENS are derived from local sensory receptors, the central nervous system, enterochromaffin cells and immune/inflammatory cells including mast cells. Enteric mast cells use the power of the immune system for detection of antigenic threats and for long-term memory of the identity of the specific antigens. Specific antibodies attach to the mast cells and enable the mast cell to detect sensitizing antigens when they reappear in the gut lumen. Should the sensitizing antigen reappear, mast cells detect it and signal its presence to the ENS. The ENS interprets the mast cell signal as a threat and calls-up from its program library secretory and propulsive motor behavior that is organized to rapidly and effectively eliminate the threat. This is an "alarm" program, the operation of which protects the individual, but at the expense of symptoms that include cramping abdominal pain, fecal urgency, watery diarrhea and threat of liquid incontinence. Enteric mast cells utilize immunological memory functions to detect foreign antigens as they appear and reappear throughout the life of the individual. Mast cells use paracrine signaling for the transfer of chemical information to the neural networks of the ENS. Integrative circuits in the ENS receive and interpret the chemical signals from the mast cells. Chemical signals from mast cells include histamine, serotonin, proteases, prostaglandins and leukotrienes. Mast cells themselves express receptors for neurokinins (e.g., NK-1 receptors) and receive input signals carried by substance P and calcitonin gene-related peptide from sensory afferents.

POST INFECTIVE IRRITABLE BOWEL SYNDROME

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The relationship between acute enteric infection and the subsequent development of Irritable Bowel Syndrome (IBS) is now well established. Indeed, gastroenteritis is the strongest risk factor identified to date for the development of IBS. The mechanisms underlying this relationship are, however, poorly understood. Increased intestinal permeability occurs in patients with post-infective IBS (PI-IBS) but its role in the development of altered motor and sensory function is unclear. It is assumed that altered permeability allows exposure of the mucosal immune system to luminal antigen, resulting in low grade immune activation and mild mucosal inflammation. The source of antigenic is likely either dietary or microbial, or both as acute infection may induce changes in gut flora. Studies in an animal model have shown that changes in gut flora result in altered sensory-motor function in the gut, and that probiotics can normalize these functions. Adequately controlled trials of probiotics in PI-IBS are awaited. The role of anti-inflammatory drugs in PI-IBS is uncertain, despite emphatic demonstrations of reversal of post-infective gut dysfunction in animals using corticosteroid or COX inhibitors. Another facet of the relationship between enteric infection and IBS is less clear. IBS is common in countries in which enteric parasitic infestation is endemic. Interestingly, eradication of the parasite does not necessarily improve IBS symptoms, generating the conclusion that the infestation and gut dysfunction are unrelated. However, studies in an animal model of chronic enteric parasitic infection suggest that this interpretation is incorrect. AKR mice are susceptible to chronic infection with Trichuris, a non invasive parasite of the cecum and chronic infection is accompanied by persistent gut dysfunction, However, while anti-helminthic treatment eradicates the infection, it does not reverse gut dysfunction. In contrast, treatment with corticosteroid after eradication normalizes gut dysfunction. Thus, lack of symptomatic improvement in IBS patients following the eradication of chronic parasitic infection cannot be construed as evidence that infection and gut dysfunction are unrelated. As is the case in acute infection, it is the immune response to the infective agent that determines gut dysfunction, and once initiated, the continued presence of the agent is irrelevant. In conclusion, on a global basis, enteric infections likely play a broader role in the development of IBS than is currently understood.

IMMUNE, CYTOKINE, AND CHEMOKINE MEDIATORS OF ILEUS

Anthony Bauer, Ph.D.
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Ileus is a major clinical problem after surgery, hemorrhagic shock/trauma and sepsis. We have been studying the molecular and cellular mechanism of ileus initiated by these three different injuries to the gastrointestinal tract. Although the triggering mechanisms for each condition are distinct, the dense network of resident muscularis macrophages appear to play a key role in the development of ileus in each of the injuries. Studies have shown that ileus is caused by an enteric molecular inflammatory response that consists of: i.) activation of the dense network of muscularis macrophages, ii.) phosphorylation of transcription factors and upregulation of cytokines, chemokines and smooth muscle inhibitory substances (iNOS and COX-2), iii.) increased expression of vascular adhesion molecules with the subsequent recruitment and extravasation of leukocytes into the circular muscle layer and the further release/secretion of various potent leukocytic products. Together these events succeed in delaying gastrointestinal transit by altering ICC and smooth muscle function, decreasing local neuromuscular transmission and activating neurogenic inhibitory pathways that suppress motility along the entire gastrointestinal tract for sustained periods. Hence, an understanding of the anti-inflammatory mechanisms of ileus are dually important in understanding the body's natural mechanisms which reign in the pro-inflammatory responses and to therapeutically exploit these mechanisms. To this end, data will be presented that explores the endogenous and exogenous therapeutic potential of the IL-10 and HO-1 pathways.

PHARMACOGENOMICS: WHAT'S HOT, WHAT'S NOT?

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Genetic factors and ultimately treatment that specifically targets these genetic factors holds the promise for a more effective treatment of patients with functional GI disorders. However, as yet, an integrated disease model for functional gastrointestinal disorders is lacking which adequately explains the broad spectrum of symptoms in functional GI disorders as well as the association with extra-intestinal syndromes and psychiatric disorders. In addition, the link between specific genetic factors and response to therapy remains to be established.

The current available data strongly suggests that there are a number of genetic factors that determine the manifestation of functional GI disorders in the presence or after exposure to specific exogenous strains. While functional GI disorders are currently stratified based upon the clinical manifestation (e.g. phenotype) or specific abnormalities of function, it can be speculated that in the future patient stratification for specific treatments has to take into account the initiating exogenous factors, the symptom pattern and the presence of protective or permissive genetic risk factors. This categorization necessitates the need to prospectively assess the link between specific abnormalities of function and genetic risk factors. While multiple genetic factors are involved, this requires large (most likely) multicenter trials that allow recruiting patients from various geographical regions with different ethnic backgrounds. This knowledge ultimately will allow much more targeted therapy.

SEROTONERGIC MECHANISMS IN FUNCTIONAL GASTROINTESTINAL DISORDERS

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The pathophysiology of functional gastrointestinal (GI) disorders is today what the actions of Russia were to Winston Churchill in 1939, "a riddle, wrapped in a mystery, inside an enigma". Clearly, there are central and peripheral components these conditions and both involve serotonin. The multiple central actions of serotonin seem to involve everything good in life but they have not been directly tied to functional GI disorders. In contrast, there is good evidence that serotonin in the GI tract contributes to the genesis of these disorders and are part of their solution. Most of the body's serotonin is in the gut; levels of serotonin in other organs are trivial in comparison. The EC subtype of enteroendocrine cell contains tryptophan hydroxylase-1 (TpH1) and secretes serotonin, which stimulates underlying nerve fibers and overflows to reach the GI lumen and blood. Because so much serotonin is secreted, and because free serotonin is toxic, uptake by GI epithelial cells, platelets, hepatocytes, and pulmonary endothelial cells inactivates serotonin. The serotonin transporter (SERT) is complemented by less avid backups. Serotonin from EC cells stimulates extrinsic nerves, which transmit sensations of nausea and discomfort to the CNS, and intrinsic nerves to initiate giant migrating contractions, peristaltic, and secretory reflexes. ENS serotonin mediates fast and slow excitatory neurotransmission. It is possible to modify serotonergic effects differentially with drugs acting at different 5-HT receptors. 5-HT3 antagonists, for example, block stimulation of extrinsic nerves and thus are useful in treating the nausea of cancer chemotherapy and the discomfort of the diarrhea-predominant irritable bowel syndrome (IBS-D). Unfortunately, 5-HT3 antagonists are constipating because they inhibit serotonergic fast neurotransmission and the activation of myenteric intrinsic primary afferent neurons (IPANs), which may initiate giant migrating contractions. 5-HT3 antagonists, however, do not block peristaltic and secretory reflexes because submucosal IPANs are activated by 5-HT1P receptors and synapses in prokinetic pathways are strengthened by 5-HT4 receptors, which increase the release of acetylcholine. 5-HT4 agonists, therefore, are useful in treating constipation-predominant IBS (IBS-C) and chronic constipation (CC). Alterations in the mucosal expression of SERT and TpH1, EC cell numbers, and serotonin secretion have been found to occur in inflammation, IBS-D, and IBS-C. These observations still have to be replicated, but the albeit limited success that has accompanied the development of agents that affect enteric serotonin has not been matched by drugs targeting other enteric neuromodulators. While it is inappropriate to consider functional GI disorders serotonergic diseases, it is fair to say that those who treat these conditions should be well aware of the enteric physiology and pharmacology of serotonin.

PSYCHOLOGICAL AND ANTIDEPRESSANT THERAPIES

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Our understanding of the irritable bowel syndrome (IBS) has changed from a dualistic "motility vs. psychiatric" disorder to a biopsychosocial model involving the interaction of dysmotility, visceral hypersensitivity, inflammation and brain-gut dysregulation in determining symptoms. This brain-body integration serves as a model for many other medical disorders.

Neurotransmitters, including serotonin (5-HT) influence motility and sensation leading to altered bowel habit and the discomfort or pain of IBS. In addition, psychosocial factors (e.g., anxiety, depression, coping style) alter brain-gut pathways and modulate these symptoms and the clinical outcome, and high levels of psychosocial distress are seen in patients with more severe or more continuous pain.

The brain registers and responds to gut function and in turn mediates the influence of psychosocial factors on gut function. This can involve alteration of mucosal inflammation/immunity via HPA axis, enteric neural signaling that leads to peripheral sensitization, and evidence from brain imaging techniques (e.g., PET, fMRI) that clarify the central pathways involved with the regulation of pain and its experience.
Centrally targeted treatments (antidepressants and psychological treatments) have been used to treat IBS for decades, but only recently is there evidence to support their benefit in this condition. The rationale for these treatments will be reviewed and include: 1) the high prevalence of co-morbid psychiatric disorders amenable to such treatments, 2) evidence that IBS patients have enhanced gut reactivity to stress (increased motility and visceral hypersensitivity), 3) cognitive bias and maladaptive coping, central mediating factors, aggravate symptoms and lead to poorer health outcomes, 4) neurophysiological evidence that central modulatory pathways in the limbic system are dysfunctional, 5) brain imaging studies that indicate improvement in the dysfunctional areas after centrally targeted treatments, and 6) evidence for clinical benefit in high quality trials of antidepressants and psychological treatments

NEUROEPITHELIUM AXIS

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The enteric nervous system is an integrative neuronal network located along the gut and is composed of both enteric neurons and glial cells. Although largely unknown, properties of enteric glial cells are thought to be similar to those of the astrocytes of the central nervous system. Intestinal epithelial barrier (IEB) is one of the major functions of the digestive tract. The concept of IEB is of major importance both in physiology and pathophysiology. As the gut stands at the interface of the organism and the luminal environment, it regulates apparently conflicting tasks such as absorption of nutrients and protection against pathogens, toxic or noxious stimuli contained in the lumen. The IEB is formed by a monolayer of polarized intestinal epithelial cells under constant turnover and held together by tight junctions.

Recent data suggest that both enteric neurons and glial cells control intestinal barrier functions such as paracellular permeability and epithelial cell proliferation via the release of neuromediators or neurotrophic factors. Furthermore, enteric glia can protect intestinal barrier from aggression by pathogens and modulate intestinal inflammation. Finally, aggression of intestinal barrier during inflammatory processes or by pathogens can modulate the neurochemical phenotype and electrophysiological properties of enteric neurons. These alterations could be responsible in part for the GI dysfunctions observed in various inflammatory pathologies such as inflammatory bowel disease or post-infectious IBS.

NEURO-INTERSTITIAL CELLS OF CAJAL AXIS

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In the gastrointestinal (GI) tract specialized cells known as interstitial cells of Cajal or ICC are distributed in specific locations within the *tunica muscularis*. At least three distinct functional classes of ICC exist. In most phasically active regions of the GI tract, a network of ICC lies within the intermuscular region at the level of the myenteric plexus and are termed ICC-MY. ICC-MY function as electrical pacemakers and also serve as a cellular network allowing for an active propagation pathway for the spread of slow waves through GI organs. A second population of ICC, known as intramuscular ICC or ICC-IM, are located within the smooth muscle layers of the GI tract and are interspersed amongst the smooth muscle cells. In the stomach ICC-IM form an intimate relationship with enteric nerve fibres and are essential for a functional cholinergic and nitrergic innervation of the smooth muscle in these tissues. ICC-IM in the gastric antrum can produce regenerative potentials and can contribute to slow wave activity in this region of the stomach. In the small intestine, ICC-IM are concentrated along the inner surface of the circular layer in the region of the deep muscular plexus, and are termed ICC-DMP. ICC-DMP are also critical for motor responses to the circular smooth muscle cells of this tissue. A further population of ICC is distributed over the surface of muscle bundles and is said to have a septal location. These ICC, known as ICC-SEP are found in the stomach, small intestine, colon and recto-anal regions of the GI tract. The physiological role of these cells has been investigated in the stomach where it has been reported that they serve much like Pukinje fibres in the heart, conveying pacemaker activity within muscle bundles which make up the wall of the stomach.

The aim of this presentation will be to highlight recent findings on the importance of ICC-IM in enteric motor neurotransmission and as integrators in stretch dependent responses in the stomach. Emphasis will be placed on the structural arrangement that exists between enteric nerve terminals and ICC-IM and between ICC-IM and smooth muscle cells that enables ICC to provide a critical mediation pathway from enteric motor nerves to effectors smooth muscle cells.

AGING AND GI SMOOTH MUSCLE: WHAT'S NEW AND WHAT'S POTENTIALLY REVERSIBLE?

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MLC_{20} phosphorylation during smooth muscle contraction is maintained by a coordinated signal transduction cascade. Transient contraction involves Ca^{2+} dependent MLCK (myosin light chain kinase)-mediated MLC_{20} phosphorylation and sustained contraction involves MLCP (myosin light chain phosphatase) dependent PKC-mediated/ RhoA/Rho-kinase-mediated maintenance of MLC_{20} phosphorylation. Reduced colonic motility has been observed in aged rats with a parallel reduction in acetylcholine-induced myosin light chain (MLC_{20}) phosphorylation. Impaired sustained contraction in aging is an outcome of defective/impaired signal transduction/signaling cascade affecting MLCP. Colonic smooth muscle cells (CSMC) from aged rat exhibited reduced translocation of RhoA, PKCα, and their association. In addition aged rat CSMC exhibited reduced translocation of myosin targeting subunit of MLCP (MYPT); decreased phosphorylation of MYPT and CPI-17. Decreased MYPT and CPI-17 phosphorylation results in activation of MLCP. Activation of MLCP results in increased phosphatase activity leading to MLC_{20} dephosphorylation which may be responsible for decreased colonic motility in aged rats. Transfection of colonic SMC from aged rats with phospho-HSP27 cDNA restored translocation of RhoA, PKCα, MYPT and phosphorylation of MYPT, and/or CPI-17, thereby restoring the contractile response similar to adult rat. Thus, we propose that phospho-HSP27 can restore sustained colonic motility in aged SMC through activation of RhoA/Rho-kinase pathway as well as PKC pathway leading to inhibition of MLCP to maintain MLC_{20} phosphorylation.

SYMPTOM DRIVEN DIAGNOSES

Yuri Saito, M.D., M.P.H.
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Symptom-driven diagnoses (SDD) represent a spectrum of disease in the field of Gastroenterology. One end of the spectrum is represented by "organic" disorders such as colon cancer or acute pancreatitis—diseases whose diagnosis depends on the presence or absence of histology, laboratory findings, or radiological imaging with less reliance on symptoms for final diagnosis. At the other end of the spectrum, there are "pure" SDDs such as functional gastrointestinal disorders (FGIDs) that are characterized and defined by the presence and absence of symptoms, because of the lack of objective diagnostic abnormalities. Diagnoses that are symptom-driven, such as FGIDs, may be relatively simple to diagnose in patients with mild symptoms seen in the primary care setting, but are much more challenging in patients with moderate to severe symptoms. The physician may ask him or herself the utility of performing further [expensive] testing, simply to prove that there is not an alternative diagnosis present. Various studies have shown that the diagnostic yield of testing in patients with FGIDs is low, and increasing reliance is being placed on symptom-based diagnostic criteria.

Over the last several decades, symptom-based criteria for FGIDs have been evolving. The most recent diagnostic criteria—referred to as "Rome III" criteria—were recently published this past spring and will be increasingly used clinically and in the research realm. The subjectivity in data collection modalities—either by the clinical practitioner or patient report in questionnaires—further complicates the diagnostic process. Nonetheless, several studies support the validity of these criteria as applied to patients. However, further testing for the discrimination of these criteria in distinguishing FGIDs and other mimickers such as celiac disease or inflammatory bowel disease requires further study.

Other special challenges in SSDs such as FGIDs include identifying the optimal primary outcome in treatment trials that captures drug "response". Depending on the perspective, "response" may be defined in numerous ways including symptom severity measures, changing from meeting diagnostic criteria to no longer meeting diagnostic criteria for disease, "adequate response", general health-related quality of life measure, disease-specific quality of life measures—to name a few. These issues in defining the optimal method for diagnosis or monitoring response to therapy are not trivial since these disorders are common, and requires considerable study to ensure that patients are not being over-diagnosed or under-diagnosed based on method of diagnosis.

IMAGE ANALYSIS: A NEW PERSPECTIVE ON FUNCTION

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Advancement in knowledge often builds upon technological progress. Conventional methods for measuring human digestive motor function (manometry, EMG, marker displacement analysis, others) that have been applied for several decades have probably already yielded much of their informative potential.

This presentation will focus on two new technological approaches currently subject to clinical research in our laboratory: intraluminal image analysis by capsule endoscopy and total abdominal volume, shape and content analysis by new CT scan software. The presentation will describe the rationale, conceptual basis, technological features and validation for these methods. It will also include preliminary data on their application to the diagnosis of patients with severe intestinal motor disorders and some functional conditions.

HISTOPATHOLOGY IN DISORDERS OF GASTROINTESTINAL MOTILITY

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Gastrointestinal (GI) motility disorders encompass a wide array of conditions, such as achalasia, gastroparesis, intestinal pseudo-obstruction, colonic inertia and megacolon. Despite recent advances, the pathophysiology of GI motility disorders remains largely unknown. A better knowledge of the pathogenetic mechanisms underlying these diseases is needed to optimize patient's management and develop new pharmacologic strategies. Tissue analysis of patients with severe forms of gut dysmotility may help elucidate abnormalities perturbing the structural and functional integrity of the enteric neuromuscular layer. Traditionally, the histopathology of GI motility disorders has been a frustrating experience amongst pathologists. Renewed interest in gut neuromuscular pathology has been fuelled by the availability of full thickness biopsies obtained with minimally invasive surgical techniques, improvement in tissue preservation, and refinement of morpho-functional techniques. Pathological abnormalities underlying GI motility disorders can be classified into three major entities: neuropathies, ''mesenchymopathies'' (i.e., changes in interstitial cells of Cajal network), and myopathies. Inflammatory/immune mediated neuropathies are characterized by either a predominant T cell (CD4 and CD8 lymphocytes) or eosinophilic infiltrate within the myenteric plexus. Other forms of neuropathies are characterized by loss of neurones together with evidence of neurodegenerative aspects in the absence of an identifiable inflammatory response. Abnormalities of interstitial cells of Cajal and smooth muscle cells may also contribute to gut dysmotility. Although the enteric neuromuscular pathology needs further study before an effective nosology can be proposed, carefully assessed individual cases and small series may provide the conceptual framework for standardizing the histological evaluation of tissue obtained from such patients. Combined clinical and histopathological studies will open new perspectives in the understanding and management of GI motility disorders.

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EPIDEMIOLOGY AND RESEARCH METHODS

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Epidemiology is the study of determinants of health and illness in individuals and populations and can allow identification of important risk factors predisposing them to a particular condition (genetic, environmental and behavioral) or assessment of interventions that might delay or prevent the onset of a disease. Once a disease is manifest, the optimal diagnostic algorithm can be tested, as can best treatment and implementation strategies for clinical practice, using an 'evidence-based' approach (which includes patients' values and preferences).

In studying a particular condition, defining and operationalizing what constitutes a case (case ascertainment) is of paramount importance. Population rates and the changes in these rates over time or across populations (geographic, socioeconomic etc.) can then be evaluated. Examples include the incidence (number of new cases diagnosed annually), prevalence (total number of cases in a population at a specified time point) and mortality rates (number of deaths in a population with a particular condition over a specific time interval, usually annually).

Epidemiologic research methods are frequently classified as observational, analytic (aimed at testing associations), and experimental (clinical trials). Observational studies are descriptive clinical observations in a single patient or patient group describing the disease course and can help generate hypotheses (of causation or treatment). Analytic studies may be cross-sectional (e.g. case-control study) or prospective (cohort study) and estimate risk using an odds ratio (between populations) or relative risk (over time), respectively. Experimental studies include clinical trials of drugs, surgeries or other interventions (e.g. behvioral, such as cognitive behavioral therapy) and ideally are both randomized and controlled. Data collection and analyses might use existing data, collected for other reasons (e.g. from government, health care or private industry databases; or pool data from a group of clinical trials for meta-analyses), by survey (e.g. postal, paper, telephone or electronic), prospectively (usually at multiple time points in natural history studies or clinical trials) and can be collected by self-administration (by the patient), interviewer or proxy respondent (e.g. family member). Such methods may have different degrees of accuracy and sources of bias (systematic error). Interpreting the results, study limitations and further questions generated are rewarding and challenging outcomes of any study. Examples will be given for gastrointestinal motility conditions.

MEASUREMENT OF QUALITY OF LIFE AND IMPACT OF THERAPY

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VA Greater Los Angeles Healthcare System
Director, UCLA/VA Center for Outcomes Research and Education (CORE)
David Geffen School of Medicine at UCLA

This talk will cover the importance of measuring health related quality of life (HRQOL) in functional GI disorders. The discussion will better enable participants to: (1) understand the concept of HRQOL; (2) recognize why knowing about HRQOL is important in clinical practice; (3) understand how HRQOL is measured; (4) know how HRQOL in FGIDs compares to HRQOL in other medical disorders; and (5) learn how to estimate HRQOL in everyday clinical practice to help direct care.

OUTCOME ASSESSMENT IN CLINICAL TRIALS

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There is good evidence that poor quality studies tend to overestimate treatment effect sizes in treatment trials of Functional GI Disorders. One of the problems in study design is the choice and validation of outcome measures. The ROME II and III working parties on Design of Clinical Trials have recommended that a global measure, which captures the relevant aspects of a particular GI disorder, is used as the primary outcome measure (1,2). There is no consensus as to how this should be operationalized in practice. A global outcome measure can either be a single outcome measure which captures overall FGID health related quality of life, or alternatively a summary score of a validated disease specific questionnaire. Fortunately much progress has been made over the last five years as increasingly well designed studies with large sample sizes have been carried out both in IBS and functional dyspepsia.

Both adequate and satisfactory relief of IBS symptoms have been used in IBS trials, evaluating alosetron and tegeserod, but as the ROME III work party has indicated, these outcome measures require additional validation. Problems with these outcome measure include, 1) potential loss of information by using a dichotomous outcome, 2) problems in considering patients equally, if they responded on having adequate or satisfactory relief but may have had different baseline levels of symptom severity, 3) inability to determine magnitude of change in severity of symptoms thereby making it difficult establishing what is the minimal clinically important difference.

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ANIMAL MODELS OF OBESITY: OF MICE AND MEN

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University of Michigan Health System

The complexity in studying the causes and treatments of obesity arises from the interaction of a variable environment with diverse genetic backgrounds displayed by an individual. Nutritional exposures in the pre-and perinatal and perinatal period have long term consequences have lasting effect on final body weight through alterations in food intake and energy expenditure. Thus, nutritional imprinting may be a significant contributor to obesity in a nutrient rich environment. As individuals age, the genetic background plays an increasingly important role in determining body weight. In order to control these factors, a host of animal models of obesity have been developed to control for one or the other of these factors. These animals have inherent advantages that increase the potential to find disease genes: they have reasonable reproduction times, their genomes have largely been sequenced, their environment can be strictly controlled and they are amenable to genetic manipulation. The three general models, spontaneous mutations, targeted mutations and diet induced obesity are used interactively to gain insight into the causes of obesity. For the most part, when compared to humans with similar monogenic forms of obesity (such as mutations in leptin or MC4R), these rodent models faithfully recapitulate the human disease. However, the significantly higher metabolism in rodents as compared to humans and differences in the formation of key tissues (such as brown fat) and proteins (β3 adrenergic receptors) highlight the distinct nature of obesity development or resistance in humans and rodents. These differences also raise the difficulty in predicting the human response of drugs developed to target obesity when screened in rodents. The emphasis on inbred lines lessens the ability to identify important modulating genes that could have important effects to modulate the impact of any single mutation. Thus, newer models of obesity and the metabolic syndrome in rodents with mixed genetic backgrounds may provide additional insights into the tissues and the metabolic pathways that contribute to susceptibility or resistance to obesity and provide metabolic targets for interventions to modulate the cause or consequences of obesity.

PERIPHERAL MECHANISMS IN THE REGULATION OF APPETITE AND OBESITY

James A. Levine, M.D., Ph.D.
Mayo Clinic College of Medicine

Non Exercise Activity Thermogenesis (NEAT) is the energy expenditure of all physical activities other than volitional sporting-like exercise. NEAT includes all the activities that render us vibrant, unique and independent beings such as working, playing and dancing. Because people of the same weight have markedly variable activity levels, it is not surprising that NEAT varies substantially between people by 2000 kcal/day. Evidence suggests that low NEAT may occur in obesity but in a very specific fashion. Obese individuals appear to exhibit an innate tendency to be seated for 2 ½ hours per day more than sedentary lean counterparts. If obese individuals were to adopt the lean 'NEAT-o-type', they could potentially expend an additional 350 kcal/day. Obesity was rare a century ago and the human genotype has not changed over that time. Thus, the obesity epidemic may reflect the emergence of a chair-enticing environment to which those with an innate tendency to sit, did so and became obese. To reverse obesity therefore, we need to develop individual strategies to promote standing and ambulating time by 2 ½ hours per day but also re-engineer our work, school and home environments to render active living the option of choice.

CENTRAL MECHANISMS IN THE REGULATION OF ENERGY AND APPETITE

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The central nervous system has begun to be recognized as an organ system critical both in the detection of central nutrient availability and in the execution of regulatory behavioral and autonomic responses that help determine energy balance. Recent work has focused on the evaluation of the ways in which central manipulation of nutrient availability alters food intake and glucose homeostasis. Effective manipulations include central administration of individual macronutrient stimuli, adiposity hormones that reflect nutrient availability, such as insulin and leptin, as well as pharmacological modulation of nutrient metabolism in discrete central nervous system regions. Recent findings include: 1) central administration of reversible or irreversible pharmacological fatty acid oxidation inhibitors acutely reduces food intake and body weight, 2) hepatic vagotomy blocks the ability of central fatty acid oxidation inhibitors to suppress glucose production but not food intake or body weight, and 3) central KATP channel inhibition blocks the ability of central fatty acid oxidation inhibitors to suppress glucose production but not food intake or body weight. Based upon the present data, we hypothesize that hypothalamic sites integrate multiple nutrient and hormonal signals regarding nutrient availability. In response to changes in nutrient status, these hypothalamic sites activate distinct central nervous system effector pathways and neurochemical signals that mediate two complimentary regulatory responses to increases in hypothalamic nutrient availability; one via hypothalamic K_{ATP} channels and hepatic vagal efferents mediating the suppression of glucose production, and the second requiring neither of these pathways in the control of food intake. Because nuttrient sensing mechsnisms are also present in the caudal brainstem, it is likely that these contribute significantly to the integrated control of food intake and energy availability as well.

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APPETITE AND OBESITY: THE GASTROENTEROLOGIST PERSPECTIVE

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Support: DK67071
Disclosure: Part of Mayo team involved in Enteromedics' program for treatment of obesity

The objectives of this review are to update the audience on perspectives of appetite control and obesity that are of relevance to gastroenterologists:

1. Factors controlling appetite
2. Gut hormones in control of appetite and upper GI motility
3. Stomach function and satiation in obesity
4. Fat absorption
5. Stomach as a target for therapy: Pharmacological (orlistat, sibutramine, cannabinoids), Devices and Surgical therapy

The main conclusions from the current analysis are:

a. The Stomach is important in integrated response to feeding, signaling satiation and appetite
b. Gastric capacity is increased in bulimia not in obesity. BMI influences maximum intake irrespective of BMI
c. The role of satiety hormones (CCK, insulin, leptin, ghrelin, PYY, GLP-1) are increasingly understood and their potential is being developed as therapeutic targets
d. Stomach volume (fasting) and emptying (accelerated at 1 h or delayed at 4 h) influence postprandial symptoms such as satiation, fullness
e. The stomach is a target for treatment: pharmacological effects of orlistat are targeted exclusively at fat absorption; sibutramine, dronabinol (cannabinoid agonist) delay gastric emptying. Novel treatments such as the experimental CB_1 receptor agonist, rimonabant, may affect stomach function. Devices (electrical, endoscopic) are being developed

GI Surgery is still the most effective approach for those with significant obesity.

Gastroenterologists, and GI surgeons with interest in motility, satiation and sensation, and clinical pharmacology or endoscopy should be at center stage in the research and clinical practice of the management of obesity.

MODELING COMPLEX BIOLOGICAL SYSTEMS: WHERE ARE WE HEADED?

Leroy Hood, M.D., Ph.D.
Institute for Systems Biology

The realization that biological systems are enormously complex and the complete genetics parts list provided by the human genome project has catalyzed the emerging field of systems biology. I will discuss how I view systems biology, the fact it is generating the view that biology is an informational science, and a systems application to an infectious disease - prion disease in mice. These studies have revealed the power of a systems approach to disease and how it can fundamentally change our view of diagnostics, therapy and even prevention. Moreover, the systems approach to disease together with new in vitro and in vivo measurement and imaging tools are catalyzing an emerging revolution in medicine where the current reactive approaches will be replaced with a predictive, preventive, personalized and participatory (P4) medicine that will emerge over the next 10-20 years. I will discuss this P4 medicine and what it means for
the health care system.

2006 Joint International Society Meeting in Neurogastroenterology and GI Motility

Seaport Hotel and World Trade Center, Boston, Massachusetts

September 14–17, 2006

Abstracts 1–30 were presented as oral presentations

Abstracts 31–405 were presented as posters

Neurogastroenterol Motil (2006) **18,** 663–798 doi: 10.1111/j.1365-2982.2006.00826.x

EDITORIAL

2006 Joint International Society Meeting in Neurogastroenterology and GI Motility

I hope many of the readers of Neurogastroenterology and Motility will be attending the Neurogastroenterology and Motility 2006 Joint Society Meeting being held September 14–17, 2006 in Boston, Massachusetts.

The American Motility Society (AMS) is the host for this inaugural meeting which is a joint venture for the four societies – the AMS, European Society of Neurogastroenterology and Motility, the Functional Brain-Gut Research Group, and the International Group for Neurogastroenterology and Motility. The Steering Committee for this meeting was chaired by Chung Owyang who was instrumental in bringing this meeting, with participation of each of the four societies, to reality. The Steering Committee included Fernando Azpiroz, Enrico Corazziari, Jean Fioramonti, Richard Locke, Juan Malagelada, Michael Schemann, Jim Galligan, and Joseph Szurszewski.

The program for this meeting has been designed to be timely, exciting and informative. The program will showcase cutting-edge research covering a broad range of preclinical and clinical topics in this rapidly expanding field, from receptors to treatment and impact on health care delivery. The organizers have made great efforts to integrate the major themes of the meeting into sessions that will be relevant and useful to a broad range of attendees: cell biologists, neurophysiologists, behavioral psychologists and psychiatrists, and clinicians. The meeting promises a good interaction and exchange of ideas with others, especially for young people deciding whether neurogastroenterology and GI motility are areas they wish to pursue. The program was constructed by the planning committee which consisted of representatives of each society. I want to thank the Program Selection Committee which was chaired by John Wiley and included Gianrico Farrugia, Jean Fioramonti, Juan Malagelada, Emeran Mayer, Michael Schemann, Vincenzo Stanghellini, and William Whitehead.

Sessions and Symposia for the meeting are on a variety of relevant topics for investigators in our field. These include: 1) New Concepts in Neurogastroenterology: Cells to Integrated Systems; 2) Role of Immune Modulation in the Brain-Gut Axis; 3) Novel Therapeutic Management of GI Motility and Functional GI Disorders; 4) Regulation of Appetite and Obesity; 5) Electrical Stimulation and Pacing of the GI Tract; 6) Novel Molecular Targets and Their Role in GI Symptoms; 7) GI Motility Disorders in Children and Adolescents; 8) Novel Signaling Pathways in the GI Tract; 9) Novel Approaches to Diagnosis; and 10) Methodologies for Health Care Research. Jim Galligan has developed an excellent program for young investigators on techniques to study GI motility at the bench and in patients.

As this meeting developed, it became apparent that this type of joint society meeting is a great opportunity for investigators to come together from around the world to attend specialized workshops for their area of interests. There are several workshops being offered on Thursday September 14, 2006: 1) Young Investigator's Session on Approaches to Study the Enteric Nervous System; 2) Role of Serotonin in GI Motility and Functional Bowel Disorders; 3) Brain Imaging and Neurogastroenterology; 4) Standardization of the Gastric Emptying Test; and 5) Understanding the Placebo Response in Clinical Practice and Trials. Immediately after the meeting there is an elective workshop on Sunday afternoon September 17, 2006 on Cyclic Vomiting Syndrome in Adults.

The joint society meetings for neurogastroenterology and GI motility are getting off to an excellent start in Boston with 404 abstracts being presented: 30 oral presentations and 374 poster presentations, allowing ample opportunity for interaction among basic scientists, clinical investigators, and others. The Boston meeting will be a model for our societies to follow for future meetings.

Henry P. Parkman, MD
President, American Motility Society

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Abstracts

1

Hydrogen sulfide: mode of action of a novel neuromodulator in the human and guinea-pig gut

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Hydrogen sulfide (H_2S) has been advocated as a mediator in the cardiovascular system and the brain. In this study, we investigated a potential neuromodulatory role of H_2S in the colon using several methods. First, we performed immunohistochemistry to explore the distribution of the H_2S-producing enzymes cystathionine γ-lyase (CSE) and cystathionine ß-synthase (CBS) in the gut wall. Second, H_2S effects on mucosal secretion were tested with Ussing chambers. Third, neuroimaging with voltage-sensitive dyes and Ca++ imaging were used to demonstrate the effect of H_2S on neuronal activity of enteric neurons. CSE–immunoreactivity (IR) was seen in practically all submucous (99%) and myenteric (96%) neurons of guinea-pig colon and submucous neurons (96%) of human colon as well as in interstitial cells of Cajal indicated by the co-localization with c-kit antibody (3 patients/animals, 20–25 ganglia each). All CSE-IR myenteric and submucous neurons were also CBS-IR and vice versa. Application of the H_2S donors NaHS (0.1–2.5mM) and L-cystein (1mM) on submucosa/mucosa preparations of guinea-pig (n=4–10) and human colon (n=5–20) resulted in a significant and dose-dependent increase (EC50=0.4mM) in ion secretion which was due to chloride secretion as demonstrated by its bumetanide (100μM) sensitivity. NaHS-induced responses were neurally mediated because tetrodotoxin (0.5μM), capsaicin desensitization (10μM) and the vanilloid receptor antagonist capsazepine (10μM) significantly inhibited secretion; L-cystein effects were significantly reduced after capsaicin desnsitization. Accordingly, NaHS (1mM) did not evoke secretion in the colonic epithelial cell line T84. Neuroimaging revealed that NaHS (0.5mM) evoked spike discharge in 23% of guinea-pig (86 neurons) and 36% of human submucous neurons (55 neurons). Spike frequency almost increased 5-fold and was significantly reduced by capsaicin desensitization and capsazepine. In contrast, NaHS (0.2–1mM) did not evoke Ca++ mobilization in cultured guinea-pig myenteric neurons arguing against a direct activation of enteric neurons by H_2S. These results indicate that H_2S may be synthesized in enteric neurons and acts as a pro-secretory mediator in the gut by activating TRPV1 receptors on sensory afferents which in turn activate enteric neurons.

2

Identification of mechano-nociceptor endings in the gastrointestinal tract

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Extrinsic sensory neurons to the wall of the gastrointestinal tract mediate all sensations arising from the gut and also activate extrinsic reflex pathways. The endings of high threshold mechano-nociceptors have not been characterized morphologically to date

Small preparations of ileum were taken from humanely killed guinea pigs and studied *in vitro*, with mesenteric membranes attached. Extracellular recordings were made from fine mesenteric nerve trunks. Mechanosensitive sites (''hotspots'') were localized with von Frey hairs (300–1000mg) and marked on the tissue. Biotinamide was applied to the nerve trunk to reveal axons that projected close to ''hotspots

''Mesenteric'' and ''serosal'' afferent endings have previously been shown to include mechanonociceptors. In the mesentery, most nerve trunks contained only bundles of smooth axons-of-passage. The only axonal specializations were ''varicose branching axons'' (VBAs) located exclusively on mesenteric blood vessels (85% on arteries, 15% on veins). Application of stiff von Frey hairs (>500mg) activated these sites in a repeatable fashion. Biotinamide filling revealed no axonal specialisations in the serosa, but VBAs were present in myenteric ganglia, circular muscle and submucosa. Von Frey hairs did not activate ''hotspots'' in serosa, myenteric ganglia or circular muscle, but revealed many in the submucosa when this was left intact. These hotspots were exclusively located on submucosal blood vessels. Strong stretch (by applying a 10g load) powerfully activated afferents with hotspots in the submucosa, but rarely activated afferents in the serosa, circular muscle or myenteric ganglia. Identical stretch applied to the mesenteries occasionally evoked weak responses in afferents with ''hotspots'' on mesenteric blood vessels. Immunohistochemical labelling identified large populations of CGRP-immunoreactive nerve endings at mesenteric and submucosal hotspots, of which >87% also contained TRPv1 immunoreactivity. The majority of submucous (>90%) and mesenteric (>66%) hotspots responded to local capsaicin (1μM).

Mechanonociceptors to the gut wall transduce mechanical stimuli at ''varicose branching axons'' located exclusively on large blood vessels. Serosal afferents, which are actually located in the submucosa, are a major source of nociceptive sensory signalling during noxious distension, and also activated by capsaicin.

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3

Ca^{2+} sensitization-mediated contraction in aged guinea pig gallbladder

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Aging impairs gallbladder function as the result, at least, of the reduction in the myogenic response to contractile agonists. The intracellular molecular events regulating decreased smooth muscle contractility during ageing still remain poorly understood. Melatonin treatment exerts beneficial effects in gallbladder contraction that are not related to changes in Ca^{2+} homeostasis. In this work, we tested the hypothesis that during ageing there is a reduction in the contribution of the RhoA/ Rho-kinase (ROCK) and PKC pathways to smooth muscle contraction via Ca^{2+} sensitization. In addition, we investigated whether melatonin enhances gallbladder contractility through this mechanism. Animals were grouped according to age in newborn (10 days old), young adults (4 months old), and senescents (20 months old). A group of senescent animals were treated with 2.5 mg/Kg/day of melatonin for 28 days. Isometric tension changes in response to agonists in the absence and presence of inhibitors of both pathways were recorded from guinea pig gallbladder muscle strips. Levels of RhoA, ROCK I, ROCK II, PKC, MYPT, MYPT-P, CPI-17 and CPI-17-P protein expression were determined by western blot. Maximal contractions in response to bethanechol and CCK were similar in newborn and senescent tissues but significantly lower than in adult and melatonin treated senescent strips. Y-27632 (5μM), an inhibitor of ROCK, reduced bethanechol-induced responses by 40–50 % in adult and melatonin treated strips, by 30% in senescent strips and had no effects on newborn tissue. Similar results were obtained in the presence of GF 109203X, an inhibitor of PKC, although the inhibition on senescent animals was smaller (18 %). Challenge of strips with CCK in the absence and presence of Y-27632 and GF 109203X yield almost identical results. Interestingly, KCl-induced responses were inhibited to a similar extent in all groups (50% and

25% of inhibition by Y-27632 and GF 109203X, respectively). In agreement with these results, protein expression of RhoA, ROCK I and II, PKC is almost inexistent in newborn and is decreased in senescent tissues. No significant differences were appreciated in constitutively expressed MYPT, MYPT-P, CPI-17 and CPI-17-P. These results suggest that Ca^{2+} sensitization mechanisms are developmentally regulated in guinea pig gallbladder and that melatonin can be used to increase Ca^{2+} sensitization in ageing. Supported by MEC (BFU 2004-0637) and JEX (2PR03A020).

4

Nodose ganglia neurons demonstrate glucose-excitatory and glucose-inhibitory responses mediated via potassium channels

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It was well known that hyperglycemia inhibits gastric motility whereas hypoglycemia stimulates counter-regulatory sympathoadrenal responses by vagal afferent pathways sensitive to capsaicin. We therefore hypothesize that glucose sensitive neurons responsive to hyper- and hypoglycemia are present in the nodose ganglia. Immunocytochemistry studies were performed on neurons isolated from rat nodose ganglia. Immunoreactivities of glucotransporters (Glut 1–4) were detected in a subgroup of neurons which also demonstrated immunoreactivities of the inwardly rectifying K+ channel 6.2 and the sulfonylurea receptor, as well as glucokinase immunoreactivities. Similar findings on gene message were obtained from RT-PCR. Whole-cell patch clamp recordings were done to determine if the excitability of vagal afferent neurons is modulated directly in response to changes in glucose concentration. In 31/113 (31%) neurons examined, increase in glucose concentration from 5 to 15 mM in the perfusing medium depolarized membrane potential (11 ± 5mV) and increased membrane input resistance (from 314 ± 47MΩ to 410 ± 46MΩ). Responses were abolished reversibly by administration of tolbutamide, a specific blocker of the sulfonylurea receptor of ATP-sensitive K+ channels. In 18/113 (15%) neurons, increase in glucose concentration was associated with membrane potential hyperpolarization (−13 ± 4.6mV) and a decrease in membrane resistance from (331 ± 66MΩ to 223 ± 27MΩ). The current-voltage plottings for 5 and 15 mM glucose concentrations crossed at −103 ± 4.6mV which corresponds to K+ ion reversal potential at the experimental conditions recorded, suggesting that activation of K+ ionic current mediated the inhibitory action of the high glucose concentration. This response was not affected by tolbutamide. The inhibitory effect of high glucose concentration was mimicked by intracellular administration of phosphadylinosotol biphosphate (PIP2). These results provide the first demonstration that vagal nodose ganglia contain both glucose excitatory (GluE) and inhibitory (GluI) neurons. In GluE neurons, glucose increases ATP production and modulates K_{ATP} channel which determines the rate of cell firing. In GluI neurons, the inhibitory effects of high glucose concentration is mediated by a K+ channel which may be related to the phosphadylinosotol pathway but not sensitive to ATP.

5

Occult reflexes that inhibit propulsion and promote accommodation in the colon are elicited by intrinsic mechano-sensitive neurons that respond to longitudinal stretch

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We have recently shown that maintained circumferential stretch of the guinea pig distal colon activates ongoing activity in ascending excitatory and descending inhibitory nerve pathways that give rise to oral EJPs that are coordinated with anal IJPs in the circular muscle (CM). This ongoing peristaltic reflex activity is driven by mechanosensory S-type interneurons that are activated by circumferential stretch rather than muscle tone or contraction. **Aims:** Although circumferential stretch is known to generate peristalsis the role of longitudinal stretch has been relatively neglected. Therefore, we investigated whether ongoing peristaltic activity generated by circumferential stretch is also affected by longitudinal stretch. **Methods:** Guinea-pigs were euthanized according to NIH guidelines. A segment of distal colon excised (20mm length). The mucosa and sub mucosa were removed and the colon mounted (serosal side uppermost) in an organ bath perfused with oxygenated Krebs' solution at 37^0C containing nifedipine (1μM) to paralyze the muscle. Simultaneous intracellular recordings were made from pairs of CM cells at either cut end (20mm apart) under maintained circumferential followed by the addition of longitudinal stretch. **Results:** The ongoing discharge of peristaltic reflex activity (oral EJPs coordinated with anal IJPs) evoked by circumferential stretch was inhibited and abolished by 25% and 60% longitudinal stretch respectively. In addition, during longitudinal stretch the polarized reflex evoked by TNS was also reduced. Ongoing reflex activity and TNS evoked activity returned to normal when longitudinal stretch was removed. Importantly, L-NA (10 μM), which is an inhibitor of nitric oxide (NO) synthesis, also completely reversed the suppression of ongoing reflex activity and TNS evoked activity by longitudinal stretch when added to a middle chamber separating the two recording sites. Also, fluid distension caused elongation of the colon that was accompanied by reduced spontaneous activity. **Conclusions:** 1) Longitudinal stretch inhibits ongoing peristaltic activity produced by circumferential stretch and polarized nerve responses to TNS. 2) Longitudinal stretch activates yet another, intrinsic, stretch sensitive mechano-sensory neuron that inhibits intrinsic peristaltic circuitry; 3) This sensory neuron is likely to be a NOS positive descending interneuron that releases NO to inhibit reflex circuitry. 4) This ***occult reflex*** (hidden from view) activated by longitudinal stretch is likely to be a powerful mechanism for retaining colonic contents.

6

A new methodology to identify central processes involved in visceral pain processing

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Recently, brain imaging studies in patients with functional gastrointestinal disorders suggest that abnormalities in brain-gut communications serve as the major contributing factor in the development of the symptomatology. To date, studies using animal models to investigate brain-gut communication have relied on post-mortem analytical techniques to determine brain areas associated with colonic hypersensitivity. The goal of the current study was to investigate whether functional magnetic resonance imaging (fMRI) could be used to identify the importance of specific brain sites in the regulation of colonic sensitivity in an animal model of irritable bowel syndrome (IBS) characterized by anxiety and colonic hypersensitivity. **Methods:** Rats were stereotaxically implanted with bilateral micropellets of either corticosterone (CORT, n = 6) to induce anxiety and colonic hypersensitivity or cholesterol (CHOL, n = 6) to serve a control. Seven days after the surgery, brain activation was investigated using fMRI. Following anesthesia and placement in the magnet (7 T), rats were subjected to a series of phasic, low-pressure colonic balloon distensions (90s off, 30s on, 40 mmHg, 8 replicates) synchronized to the triggering of the magnet. Cross-correlation statistical analysis was used to determine significant differences between distended and non-distended states in CORT and CHOL treated animals. **Results:** Colonic distension (40 mmHg) induced greater overall brain activation in rats with CORT implants compared to CHOL controls in specific nuclei know to be involved in anxiety (amygdala, hippocampus) and pain perception (thalamus). **Conclusion:** This is the first study to show that differences in brain activation exist in a rodent model of anxiety and colonic hypersensitivity, which represents major characteristics of IBS.

7

Temporary percutaneous gastric electrical stimulation (GES). A new technique to test response to GES

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GES has proven to be effective for drug refractory nausea and vomiting in diabetic and idiopathic gastroparesis (IGP). However, the proportion of non-responders is reported high, up to 50%, particularly for IGP. Moreover, the spectrum of indications for GES and optimal stimulation (stim) settings are yet to be explored. We introduce a new percutaneous technique and have tested the response to GES in patients (Pts) with drug refractory nausea and/or vomiting and non-established indications for GES. **Methods:** Pts with drug refractory symptoms (functional dyspepsia, FD, 7 pts; chronic intestinal pseudobstruction, CIP, 3 pts; postsurgical gastroparesis, PS-GP, 4 pts) were implanted at gastroscopy with two percutaneous electrodes anchored to the gastric submucosa. The leads were connected to an Enterra (Medtronic) impulse generator taped to the abdomen during the test. Seven pts had an open stim for 7–21 days with standard settings (12 imp/min, 5 mA). Seven pts were, after implantation, randomized to double-blind crossover stim with standard settings (stim ON for 10–14 days, OFF for 10–14 days). Symptoms were recorded daily. Pts without a clear-cut response were offered a further period with open stim at 8–10 mA. **Results:** Implantation time for the two leads from the start of gastroscopy was 10–22 min (mean 15 min). Leads were kept implanted for up to 8 weeks without complications. Endoscopy at the end of the test revealed no lead related lesions. Eleven of the 14 pts had a favourable decrease of the referral symptoms nausea/vomiting: FD 6/7 pts, PS-GP 3/4 pts, CIP 2/3 pts. Three pts had a marked symptom reduction (>80%) during the ON period compared with stim OFF. Two pts improved when stim was increased from 5 to 8 mA. Improvement was independent of distance between electrodes: marked improvement seen from 0 mm (monopolar stim, 1 pt) up to 60 mm electrode distance. The three non-responders (1 FD; 1 PS-GP; 1 CIP with lack of ICC) did not respond to stim with 8–10 mA. In 10 of the 11 responders a decision for permanent GES implant could be made. In one pt (myopathic CIP) the nausea disappeared during GES but permanent implant was not done due to persisting intestinal failure. **Conclusion:** Percutaneous temporary GES seems to be a promising principle to study new indications for GES and to select responders/non-responders. The optimal stim settings for double blind tests need to be further studied.

8

Central neuronal mechanisms of gastric electrical stimulation and alterations in central neuronal activities in obese rats

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Recent brain imaging has revealed altered central metabolic activities related to food intake in obese patients. However, it is unknown whether neuronal activities in the satiety center (ventromedial nucleus or VMH) and their responses to gastric stimulations are altered in obese subjects. Gastric electrical stimulation (GES) has recently been proposed for treating obesity with mixed results; its possible central mechanisms remain largely unknown and there is a need to optimize the methodology. The aims of this study are: to study the responses of neurons in the VMH to gastric distention and GES and to investigate the effects of GES with different parameters on the VMH neurons in diet-induced-obese (DIO) rats. **Methods:** Ten lean rats (controls) and 12 obese rats induced by high-fat diet were used in the study. Under laparostomy a balloon was placed into the stomach for gastric distention and one pair of electrodes was sutured on the serosa of the antrum for GES. Single neuron activity in the VMH was recorded using the standard extracellular recording technique at baseline, during graded gastric distention (GD, at 20 and 60mmHg) and GES with different parameters (GES-0.3ms-used in treating obesity: 6mA, 0.3ms, 40Hz, 2s-on, 3s-off; GES-3ms: same as GES-0.3ms but a pulse width of 3ms). **Results:** 1) 83 neurons in the VMH were recorded from the 22 rats and 73.5% of them were responsive to GD of 60mmHg; 2) A significantly lower percentage of neurons (31.6%) were activated by GD of 20mmHg in the DIO rats, compared with the regular rats (60.8%) (P=0.048). 3) The percentage of neurons responsive to GES-0.3ms in the DIO rats was significantly lower than that in the regular rats (31.5% vs. 78.2%, P<0.03). This difference became insignificant when GES was applied with the increased pulse width of 3ms (84.6% vs. 96%, P=0.17). **Conclusions:** 1) DIO rats are less responsive to physiological gastric distention as well as GES of parameters used in clinical trials. These data suggest that DIO rats are more resistant to both gastric distention and GES. 2) GES with wider pulses may be needed to treat obesity. (This study was partially supported by Medtronic).

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Inhibitory effects of electrical stimulation of the stomach, intestine and colon on rectal tone

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Background: A phenomenon of cross-talk has been noted with electrical stimulation of the gut: electrical stimulation of one part of the gut affects another part of the gut. **Aims:** The aim of this study was to investigate whether the effect of electrical stimulation of one part of the gut on another part of the gut was related to the organ or the distance between the stimulation site and the affected organ. **Methods:** Effects of gastric electrical stimulation (GES), duodenal electrical stimulation (DES), ileum electrical stimulation (IES) and colonic electrical stimulation (CES) on rectal tone were studied in 8 healthy female hound dogs (16–23Kg) implanted with one pair of gastric serosal electrodes (2cm above pylorus), one pair of duodenum serosal electrodes (10cm below pylorus), one pair of ileum serosal electrodes (10cm above cecum) and one pair of colonic serosal electrodes (10cm below cecum) and a gastric cannula 10cm above pylorus. A computerized barostat was used to assess rectal tone by measuring the rectal intra balloon volume. Electrical stimulation was performed using repetitive long pulses (pulse width 300ms, amplitude: 10mA, frequency: 12 pulses/min). Each experiment was performed in 4 randomized sessions on 4 separate days with an interval of at least three days apart. In each study session, rectal tone was recorded for 30–40min at baseline, 25 min during stimulation and 25min at recovery. **Results:** 1) All methods of stimulations significantly inhibited rectal tone. The rectal volume was increased from 84.6±10.2ml at baseline to 124.3±20.4ml (P<0.02) with GES, from 83.9±4.9ml to 89.6±5.3ml (P<0.02) with DES, from 87.6±10.2ml to 117.8±15.6ml (P<0.006) with IES and from 89.2±9.9ml to 145.0±20.1ml (P<0.04) with CES. 2) DES was least effective in reducing rectal tone compared with any of other methods (P<0.05 vs. GES, IES or CES). The percentage of increase in rectal volume was highest with CES 60.7±11.9% although this was not significantly higher than that with GES (42.3±11.7%) or IES (34.0±5.9%). **Conclusions:** Electrical stimulation of the stomach, intestine or colon reduces rectal tone. The inhibitory effect is organ-specific and not related to the distance between stimulation site and affected organs.

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Effect of a cannabinoid receptor agonist on human colonic motor and sensory functions

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Cannabinoid receptors (CBR) are located on cholinergic neurons in the brain stem, stomach and colon. CBR stimulation inhibits motility in rodents. Effects in humans are unclear. **Aims:** To compare the effects of dronabinol (DRO), a non-selective CBR agonist, and placebo (PLA) on colonic motility and sensation in humans. **Methods:** In a double-blind, randomized, parallel-group study, 52 healthy volunteers

received DRO 7.5 mg or PLA p.o. After bowel cleansing, a barostat assembly was placed in the descending colon with the aid of sigmoidoscopy and fluoroscopy. We assessed colonic compliance and sensation (ascending method of limits, sensory ratings of gas and pain to random order distensions, 8–36mmHg) prior to and after medication. A 1000 kcal liquid meal was ingested and motility recorded for 1 hour. Planned sample size had sufficient power to detect 38% differences in primary motor endpoint (colonic tone) and 60–70% change in sensory ratings. **Results (table):** There was inhibition of postprandial colonic tone (p=0.048), increased compliance (p=0.045) and a borderline relaxation of fasting tone (p=0.096) with DRO. The effect on compliance was most pronounced in females. DRO did not significantly alter sensation thresholds. There was an increase in rating of pain during phasic distensions at all pressures and in both genders (p=0.024). **Conclusion:** In humans, DRO relaxes the colon and reduces postprandial colonic tone; increased colonic compliance with DRO is gender related. Increase in pressure-mediated sensation with relaxation of colonic function suggests central modulation by DRO and requires further study.

Data mean±SEM	Placebo (n=28)		Dronabinol (n=24)	
Age (y)	34.2±2.5		36.8±2.8	
Gender F:M	16:12		14:10	
BMI (kg/m2)	24.5±0.7		25.3±0.7	
	pre	post	pre	post
Colonic compliance, Pr half, mmHg	19.4±0.6	17.3±0.7	17.4±0.8*	15.0±0.8
Colonic fasting tone, ml	89.1±6.0	96.2±8.6	88.1±6.8	102.7±8.2**
Colonic postprandial tone, ml		64.3±6.5		88.0±9.4*
Sensory threshold gas, mmHg	25.3±3.7	29.5±3.5	20.2±3.5	27.4±3.5
Sensory rating pain 36 mmHg, mm VAS	43.6±5.0	41.5±5.2	40.1±5.0	52.8±5.6*

*p<0.05; **p<0.1

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Proteinase-activated receptor-2 (PAR-2) activation evokes esophageal longitudinal smooth muscle (LSM) contraction via a capsaicin-sensitive and neurokinin-2 (NK_2) receptor-dependent pathway

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Intraluminal acid evokes sustained contraction of esophageal LSM and esophageal shortening, which may contribute to the pathogenesis of hiatus hernia formation and non-cardiac chest pain. In the opossum, acid-induced esophageal shortening is not prevented by vagotomy or atropine pretreatment but is attenuated by mast cell stabilizers, desensitization of capsaicin-sensitive neurons or antagonism of the neurokinin NK_2 receptors. This suggests that mast cell mediators released by acid may evoke LSM contraction via release of neurokinins from capsaicin sensory neurons. Mast cell tryptase is a potent agonist for PAR-2 receptors, which have been reported to be present on capsaicin-sensitive primary afferent neurons. The objective of the current study was to determine whether PAR-2 activation also leads to sustained LSM contraction via stimulation of capsaicin-sensitive neurons and activation of NK_2 receptors. Trypsin evoked a concentration-dependent sustained contraction of LSM strips from opossum distal esophagus, but had no effect on circular smooth muscle strips. The PAR-1/3 agonist thrombin had no effect on LSM strips. The trypsin-induced contraction was unaffected by tetrodotoxin (1 μm), but was abolished by prior capsaicin desensitization (30 μm), or by pretreatment with a soy bean trypsin inhibitor (10 μm) . Preincubating the strips with the selective NK_2 antagonist MEN-10376 (1 μm) also abolished the trypsin-induced contractions. In single isolated LSM cells, trypsin failed to evoke a contractile response or an increase in intracellular calcium using Fluo-4 loaded cells. Immunohistochemical studies revealed evidence of PAR-2 receptors within nerve profiles in the mucosa and LSM layer, but not on the muscle cells. PAR-2 immunoreactivity was seen to co-localize within approximately 20% of the substance P/CGRP positive nerve profiles. These results suggest that LSM contraction induced by the PAR-2 agonist trypsin is not due to direct activation of the muscle. Rather, it involves capsaicin-sensitive neurons and activation of the NK_2 receptors, which appears to be identical to the pathway involved in LSM contraction induced by luminal acid perfusion. PAR-2 receptors may therefore play a role in the pathophysiology of reflux esophagitis. (Supported by CIHR).

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Sensory-motor abnormalities in severe gut dysmotility: role of anti-HuD neuronal antibody (A-HuD)

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The role of autoimmunity in gut dysmotility remains poorly defined. This study was designed to identify the mechanisms through which A-HuD IgG affects gut function. A-HuD IgG was isolated by affinity chromatography from sera of patients. Specificity of A-HuD IgG was verified by selective staining of enteric neurons. A-HuD-free serum (i.e., A-HuA/B/C) served as control. Four groups of experiments were performed to examine effects on enteric and visceral sensory neuronal function. First, Ca^{2+}-imaging of cultured guinea-pig myenteric neurons revealed that A-HuD (1:50), but not A-HuA/B/C IgG, increased the intracellular calcium concentration in 90% of the tested neurons (total number of 55), a response dependent on extracellular Ca^{2+}. Tetrodotoxin (0.3 μM), nifedipine (1 μM), ω-conotoxin (0.1 μM) and the receptor-operated calcium channel blocker SKF96365 (30 μM) significantly inhibited the A-HuD-induced Ca^{2+} mobilization by 91%, 73%, 76%, and 88%, respectively. Second, fast neuroimaging of LMMP preparations revealed that microejection of A-HuD (400ms, 1:20, 22 ganglia, 4 guinea-pigs) activated 37.4±8% of myenteric neurons causing an immediate spike discharge or increase in spontaneous firing lasting several minutes. Only few neurons (6±1%) were activated by A-HuA/B/C serum. Third, local perfusion of A-HuD but not A-HuA/B/C significantly increased firing in 15/16 vagal mechanosensitive fibers innervating the mouse gastric fundus. The effect peaked at 76±11s and had a rapidly desensitizing component (t1/2 10.2s) as well as a sustained response. DMPP and ATP sensitive and insensitive hotspots responded. The response was similar in calcium-depleted buffer (22.9±5.2 spikes/s vs. control 19.2±3.7). Fourth, in patch clamp studies on back-filled nodose neurons A-HuD evoked an inward current in 6/18 cells which reversed around +10mV, consistent with a non-selective cation conductance. In conclusion, A-HuD IgG isolated from patients with severe gut motility dysfunction causes activation of both enteric neurons and sensory vagal afferents. These data suggest that humoral autoimmunity activates abnormal immune-neural signaling in enteric and vagal mechanosensory neurons which play a role to sensory-motor dysfunction observed in patients with severe gut dysmotility.

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Transit studies, colonic manometry and transcutaneous electrical stimulation to diagnose and treat slow transit constipation in children

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Slow transit constipation (STC) was first described in children 10 years ago (Benninga et al. 1996 J Pediatr Gastroenterol Nutr 23:241–51). This study reports our experience at Royal Childrens Hospital, Melbourne,

using radio-isotope transit studies and colonic manometry to investigate 300 children with chronic treatment-resistant constipation and development of a treatment targeted at the colon. **Methods:** Whole gut transit was measured using gamma camera scintigraphy at 0,2,6,24,30 & 48 hrs. 24hr colonic manometry using 8 channel water-perfused catheter was performed via established appendix stomas. For transcutaneous electrical stimulation 2 surface electrodes were placed anterior & 2 posterior near the umbilicus. Pulsed currents were crossed and applied for 20 mins, 3 /wk for 4 wks. **Results:** Children with metabolic, genetic disorders, Hirschsprung's disease or palpable fecaloma were not sent for nuclear transit studies. 120 nuclear transit studies were performed. 48hr studies separated patients with normal transit (16%), anal retention (11%) and slow colonic transit (SCT, 73%). SCT consisted of 3 subgroups: pancolonic slowing (44%), splenic flexure holdup (16%) and combined small and large bowel slowing (13%). 24 hr colonic manometry was performed via the appendix stoma on children classified from transit studies as SCT (18) or non-SCT (8). SCT children had reduced frequency of anterior propagating contractions ($p<0.05$) and no increase in activity post-waking or post-meal compared to non-SCT children (4 anal retention, 4 normal transit). Transcutaneous electrical stimulation applied at the level of the umbilicus to SCT children, increased defecation (13/16) and stopped soiling (15/16). The effects lasted >3 months post-stimulation. Two patients had colonic manometry before and 6 months after electrical stimulation. In both, the frequency of antegrade propagating contractions increased into the normal range, defecation normalised and soiling stopped. **Conclusions:** 48 hr radioisotope transit studies can separate SCT from outlet obstruction. 20% of children with chronic constipation had SCT independent of outlet obstruction. SCT was associated with reduced propagating contractions in the colon and no stimulation of activity following a meal or waking. Colonic propagating activity and defecation were increased by transcutaneous electrical stimulation over the colon. The effects were long lasting and associated with an increase in intraluminal propagating activity.

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The incidence of cyclical vomiting syndrome in the paediatric population of Ireland

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Aims: Cyclical Vomiting Syndrome (CVS) is an uncommon disorder of children characterized by recurrent episodes of vomiting causing significant morbidity in those affected. We undertook a prospective national study of the incidence and presenting features of CVS through the Irish Paediatric Surveillance unit. **Methods:** All one hundred and fifty-four paediatricians on the island were surveyed on a monthly basis from January 2005 – January 2006. We collected information regarding demographics, clinical features, hospitalization, investigations and interventions. Data was analysed using Epi-Info. **Results:** There was a 91% return rate of monthly reporting cards. In all, 51 new cases were reported during the study period, giving an incidence of 3 / 100,000 new cases per anum. Forty-five questionnaires of the total 51 cases reported were returned. 46% cases were male. The mean age of onset of CVS was 4.5 years, (SD 3.4, Range 1–14years). The majority of children (53%) had been referred to the paediatric clinic by their primary care provider. The mean number episodes of CVS per child per year was 9.7 (SD 9.8, Range 1–52), with a mean duration of these episodes of 40.4 hours (SD 36, Range 1 hour to 14 days). Other functional complaints were prevalent with recurrent abdominal pain in 22% and regular non-migraine headaches in 8.9%. A family history of migraine was found in 34.1%. Of those studied, 67.6% had missed school in the previous year due to CVS. Forty percent were admitted to hospital for CVS, the majority (80%) for intravenous rehydration. **Conclusion:** While previous studies have reported population prevalence of CVS as 1.5–2.3% through cross-sectional surveys, we report the first national incidence study of clinically significant CVS. We describe presenting and associated features, while raising awareness of the disorder amongst paediatricians.

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Is visceral hyperalgesia correlated with symptoms severity in children with functional gastrointestinal disorders?

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Abdominal pain related to functional gastrointestinal disorders (FGID) is frequent in children and can be of variable severity. Functional abdominal pain (FAP) and irritable bowel syndrome (IBS) are associated with rectal hypersensitivity. Our aim was to test the hypothesis that, in children with IBS or FAP, the rectal sensory threshold for pain (RSTP) is correlated with symptoms severity. **Methods:** 52 patients (37 girls, mean age 13 years, range 8.5 to 17 y) with IBS (n=30) and with FAP (n=22), according to Rome II criteria, underwent a series of rectal isobaric balloon distensions using an electronic barostat to determine their RSTP. The patients completed the Questionnaire on Pediatric Gastrointestinal Symptoms in Children underlining symptoms of importance such as pain frequency and duration, missed days of school and missed activities with friends due to pain. **Results:** The mean RSTP of IBS patients (19 mmHg ±10) was not significantly different from that of the FAP patients (23 mmHg ±11; p=0.2). 85% of the patients had a RSTP ≤ 30 mmHg which represents the 5th percentile of our control population and were considered hypersensitive. 81% reported abdominal pain for more than one year and 91% once a week or more. 52% and 37% respectively reported missing school and social activities at least once a week. No significant association was found between RSTP and pain frequency, duration of pain, missed days of school and missed social activities in the total population (multiple logistic regression p>0.20). The subset of patients with hypersensitive values of RSTP similarly did not demonstrate any relation with the same variables (multiple linear regression p>0.25). **Conclusion:** In our population, rectal hypersensitivity, as measured by RSTP, was not proportional to the gravity of symptoms in children with IBS and FAP, suggesting that symptoms severity in FGID is not only under dependence of visceral hypersensitivity but is also influenced by other factors.

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Role of STAT4 in the regulation of functional and immune responses in infectious colitis

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IL-12 and TNFα are synthesized by macrophages upon exposure to bacterial products. STAT4 is required for IL-12 mediated Th1 differentiation. **Aims:** To determine the contribution of STAT4 to constitutive and inflammation-induced alterations in colonic smooth muscle and epithelial cell function. **Methods:** BALB/c and STAT4$^{-/-}$ mice received vehicle (Control) or live *Citrobacter rodentium (Cr)* to induce colitis. 12 days later, we determined smooth muscle (organ bath) and epithelial (Ussing chambers) responses to acetylcholine (Ach). Th1 cytokine, T-bet, and iNOS expression were determined using real time PCR. **Results:** *Cr* infection decreased both contraction and secretion in response to Ach. Cr increased iNOS, T-bet, and Th1 cytokine, but not IL-4, expression. Uninfected STAT4$^{-/-}$ mice increased, T-bet, iNOS, and Th1 cytokine expression and had reduced responses to ACH. In STAT4$^{-/-}$, Cr blunted the normal proinflammatory response and failed to inhibit further colonic function. Cr-induced elevation in iNOS and IL-12p40 were independent of STAT4 and suggest that IL-12p35 and IL-12p40 are regulated separately. **Conclusions:** *Cr*-induced hyposecretory and hypocontractile effects in the colon are associated with a Th1 profile. STAT4 exerts a dual effect on colonic function. It appears to have a paradoxical constitutive anti-inflammatory effect and modulates normal colonic function. It also mediates the smooth muscle and epithelial responses

as well as the major portion of the Th1-driven adaptive immune response to *Cr.*

	Control	Cr	STAT4$^{-/-}$	*Cr* + STAT4$^{-/-}$
Ach (N/ cm^2)	10.9±1.0	4.1±1.5*	5.6±0.8 *	7.9±1.0
Ach (μA/cm^2)	76±16	49±12*	21±10*	31±12
T-bet	1±0.7	6.4±1.0*	5.8±1.0*	2.2±0.2
IFN-γ	1±1.1	12.9±2.7*	8.2±1.9*	2.5±0.5
IL-12 p35	1±0.5	3.5±0.5*	6.7±0.9*	1.9±0.3
IL-12 p40	1±0.4	441±111*	3.5±0.7*	471±76ϕ
iNOS	1±0.8	13.6±4.9*	4.5±0.9*	8.2±1.2ϕ

P<0.05 vs VEH; ϕp<0.05 vs STAT4$^{-/-}$; mRNA expressed as a fold change from VEH

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On the mechanisms of acquired enteric neuropathies: a model of herpes simplex virus -1 (HSV1) infection of the rat enteric nervous system *in vivo*

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Enteric nervous system (ENS) degeneration may occur in digestive neuropathies responsible for severe gut dismotility. Among factors evoking ENS degeneration, neurotropic viruses are known to damage the enteric neuronal integrity. However, the mechanisms through which such viruses affect the ENS are still largely unknown. The aim of the present study was to establish an *in vivo* model of HSV1 infection in the rat ENS. **Methods:** Persistent HSV1 infection in the ENS was induced by inoculating rats with HSV1 in two occasions: first intranasally and then via intragastric gavage. Briefly, six weeks after intranasal HSV1 (10^4 PFU) instillation rats were injected intragastrically with either HSV1 (n=10) or saline (n=6). Following additional 6 weeks animals were sacrificed and the brain, gastrointestinal tract, adrenal glands, spleen, liver and lungs collected. In addition, enteric neurons isolated from small bowel myenteric plexuses were cultured. The presence and nature of HSV1 infection was studied as follows: a) HSV1 DNA was detected by PCR amplification for HSV1-TK gene; b) latency associated mRNA transcripts (LAT) were detected by RT-PCR and c) early gene ICP-4 mRNA transcript associated with active viral replication was detected by RT-PCR. Finally, viral distribution was studied by in situ hybridization and immunohistochemistry directed against HSV1 glycoprotein-C (gC). **Results:** Controls and HSV1 infected rats did not show any sign of disease (e.g., weight loss, abdominal distension, diarrhea) as well as any macroscopic and microscopic abnormality. Viral TK-DNA and LAT mRNA, but not ICP-4 transcripts, were detected in brain, duodenum, ileum, and adrenal glands by PCR and RT-PCR, respectively, indicating a chronic persistent infection in these tissues. HSV1 genome and LAT transcripts, but not gC immunoreactivity and ICP-4 mRNA transcripts, were detected also in isolated enteric neurons supporting the presence of a latent infection. By in situ hybridization we observed the presence of HSV1 gC DNA in ENS and CNS neurons. **Conclusions:** Our data indicate that HSV1 establishes a latent infection in the ENS following intragastric challenge. HSV1 latent infection may reactivate and evoke enteric neuron dysfunction/damage leading to disturbed gut motility and transit.

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Recovery role of IL-10 in postoperative ileus

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Introduction: Pro- and anti-inflammatory molecular responses within the intestinal muscularis play an important role in causing and resolving postoperative ileus. Our previous studies showed that exogenous carbon monoxide attenuated the development of ileus, and that this was accompanied by an enhanced induction of the anti-inflammatory mediators HO-1 and IL-10. Our objective was to directly examine the role of IL-10 following intestinal surgical manipulation. **Methods & Results:** Laparotomy and surgical manipulation of the murine intestine resulted in a delay in gastrointestinal transit that recovered over a period of 5 days, as measured with non-digestible FITC-labeled dextran fed orally 90 minutes before sacrifice. Basal gastrointestinal transit was not different between control wild-type C57Bl6 mice (GC=11.0) and IL-10 knock-out mice (GC=10.8); however, the temporal recovery of gastrointestinal transit after surgical manipulation was more prolonged in IL-10 knock-out mice (GC=3.8, 6.1 and 8.4, for postoperative days 1, 3 and 5, respectively) compared to C57Bl6 mice (4.4, 6.9 and 11.3, for postoperative days 1, 3 and 5, respectively) (N=5 for all groups). When the isolated muscularis of C57Bl6 manipulated animals harvested 3 hours after surgery was analyzed for mRNA expressions contained on the ABI 96 gene TaqMan Low Density Array Mouse Immune Panel, a significant upregulation was measured in numerous genes including: NF-KB, SOCS, PGHS-2, IL-1, IL-6 and HO-1. Interestingly, at this early 3 hour time point a similar pattern of mRNA expressions was measured in the manipulated IL-10 knock-out muscularis (ie. IL-6 CT values: WT control=33.01±0.383 to WT manipulated=26.76±0.353 vs. IL-10 KO control= 32.57±0.436 to IL-10 KO manipulated=26.77±0.891, N=4 each). **Conclusion:** The combined gastrointestinal transit and mRNA data indicate an important role for endogenous IL-10 in the ''recovery'' phase of postoperative ileus, rather than on altering the initiation of the proinflammatory molecular response. The expression of mRNAs at later time points is ongoing. These findings indicate that IL-10 plays a significant role in the recovery phase of the gastrointestinal tract from postoperative ileus and suggest that augmentation of the IL-10 pathway is a potential mechanism to speed recovery from postoperative ileus.

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The influence of candidate genes on the response of sibutramine treatment for weight loss in overweight and obesity

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Background: Sibutramine is a noradrenergic (NE) and serotonergic (5-HT) reuptake inhibitor approved for the treatment of obesity. Prior studies suggest that genetic variations in phenylethanolamine N-methyltransferase (PNMT) and GNβ_3 influence the weight loss response to sibutramine. **Aim:** To assess the influence of candidate NE and 5-HT genes on weight loss in response to sibutramine or placebo treatment in healthy overweight and obese individuals. **Methods:** Forty-eight healthy overweight and obese participants were randomly assigned to receive placebo or sibutramine, 15 mg daily, for 12 weeks. Venous blood DNA was analyzed for SERT-P (SLC6A4), α-2 MspI, PNMT and GNβ_3 C825T genotypes. All participants also received structured behavioral therapy for weight management. Separate ANCOVA models were used to assess each candidate gene's associations with weight loss. **Results:** The SLC6A4 genotype had a significant influence on the effect of sibutramine on the change in weight (p=0.024). Subjects with SLC6A4 LS/SS (heterozygous/short) genotype had an average weight loss of 6.1±1.0 kg on sibutramine compared to 0.1±0.9 kg average weight increase on placebo. In contrast, for subjects with the homozygous SLC6A4 LL (long) genotype, weight was similar in both treatment groups, 3.3±1.8 kg in placebo compared to 3.9 ± 1.6 kg in the sibutramine group. There were no significant associations of treatment with the α-2a MspI, PNMT, and GNβ_3 genotypes. **Conclusions:** Genetic modulation of endogenous serotonin reuptake may explain, in part, the variation in the weight reduction observed with sibutramine. This may provide guidance on selection of patients for this pharmacological treatment of obesity. Grant support: NIH RO1 DK67071 (MC).

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5-HT$_4$ receptor-immunoreactivity (5-HT$_4$-IR) is expressed in non-neuronal cells of the human gastrointestinal (GI) tract

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Introduction*:* 5-HT$_4$ agonists are pro-kinetic and used to treat patients with chronic constipation and constipation-predominant IBS. Although there are a number of studies describing the pharmacological actions of 5-HT$_4$ agonists on human GI samples, there is a paucity of data on the morphological localization of 5-HT$_4$ in the human gut. **Methods:** Samples [n] of non-involved regions of different levels of the human GI tract (Oesophagus [5], stomach [12], small bowel [8], colon [22] and rectum [2]) from patients with various disorders were obtained with consent. These tissues and rat colons were formalin fixed and paraffin embedded (FFPE), sectioned and stained using polyclonal antisera specific for either the 3rd cytoplasmic domain (LS655, LifeSpan, WA), or C-terminus of the common sequence of the splice variants (NLS656, Novus, CO). 5-HT$_4$ double-stains with glial fibrillary acid protein (GFAP, Dako, CA), smooth muscle actin (Zymed, CA), GAP-43 (Novus) and tryptase (Calbiochem, CA) were performed. FFPE wild type / 5-HT$_{4b}$-transfected CHO cells and DU145 cells were used as controls. *In situ* hybridization (ISH) using 30mer biotinylated oligonucleotide probes was also performed. **Results:** 5-HT$_4$-IR using LS655 labelled a small proportion of 5-HT$_{4b}$-transfected CHO cells, with no staining in wild type CHO cells. 5-HT$_4$-IR was localized in the muscularis mucosa, muscularis propria (circular and longitudinal) and blood vessels, in a punctuate pattern, in all tissue samples examined. Similar muscle staining was seen using ISH. Strong 5-HT$_4$-IR using LS655 was observed in tryptase-IR mast cells throughout the GI tract. However, neurons did not exhibit convincing 5-HT$_4$-IR in any sample. Similar patterns of 5-HT$_4$-IR in the GI tract were observed using NLS656, with strong staining in smooth muscle and positive mast cells (although weaker than with LS655), but not in neurons. However, using NLS656 there appeared to be peri-neuronal 5-HT$_4$-IR in both submucous and myenteric ganglia, consistent with the labelling of glial cells. The specificity of NLS656 is unclear though, as 5-HT$_4$-IR was observed in both 5-HT$_4$ transfected and wild type CHO cells. **Conclusion:** The distribution of 5-HT$_4$-IR was consistent throughout the different regions of the human GI tract, although the cellular localization varied depending upon the primary antiserum employed. The most consistent 5-HT$_4$-IR staining was on muscle cells, while neurons appeared to be negative. The labelling of mast cells, and perhaps of glial cells, suggest that in addition to a direct action on muscle, 5-HT$_4$ ligands might act indirectly through non-neuronal cell types.

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Visceral sensitivity as a mediator for the relationship between general medical anxiety and symptom severity

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Background: Anxiety about specific gastrointestinal (GI) sensations is thought to play a key role in the maintenance and perhaps development of irritable bowel syndrome (IBS) symptoms. Gastrointestinal-specific anxiety (GSA) can be defined as the cognitive, affective, and behavioral response stemming from fear of GI sensations, symptoms, and the context in which these visceral sensations and symptoms occur. As a marker of over responsiveness to GI sensations, GSA is hypothesized to perpetuate IBS symptoms through alterations in autonomic and pain facilitation, as well as cognitive mechanisms. GSA is considered distinct from anxiety sensitivity (AS) fear of anxiety itself, and general medical anxiety (GMA). Recently, the Visceral Sensitivity Index (VSI) was developed as the first instrument to assess GSA. Initial validation efforts indicate that the VSI is an efficient, reliable, and valid measure of GSA in an IBS patient population **Aims:** To examine the potential mediating effect of GSA on the relationship between GMA and AS on symptom severity in IBS. **Methods:** 96 Rome II positive IBS patients were recruited by advertisement (21 males, 75 females; mean age = 49.4) and administered measures assessing the presence of GSA, AS, GMA (Anxiety Sensitivity Index; ASI), GMA (Whitely Index), and GI-symptom severity. Path analysis via structural equation modeling framework in AMOS 6 was to test the potential mediating role of the VSI. **Results:** Bivariate correlations revealed all measures were significantly related to GI symptom severity (r=.23–.45). The VSI significantly mediated the effect of ASI and GMA on symptom severity in IBS (p's<.01). **Conclusion:** Results support GSA as a common pathway for the expression of somatization and general anxiety in IBS, and the VSI as an important measurement tool of this construct.

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Enteric glial cells (EGC) support the intestinal epithelial barrier

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Loss of EGC is accompanied by a breakdown of epithelial integrity and subsequent intestinal inflammation (Bush e.a. Cell 1998). To analyze interactions between EGC and the intestinal epithelial barrier in un-inflamed and inflamed conditions, intestinal epithelial cells (IEC, T84) were grown on filter supports in the apical compartment of a transwell co-culture system in the presence or absence of EGC in the basolateral compartment. Mature IEC were stimulated for 24hrs with human recombinant tumor necrosis factor (TNF)α (1, 10, 100ng/mL), interleukin (IL)-1β (1, 10, 100ng/mL), IL-13 (10ng/mL) or interferon (IFN)γ (100ng/mL). Subsequently, the integrity of IEC monolayers was determined by assessing the transepithelial electrical resistance (TER) over a period of 48hrs. To detect cytokine-induced defects in the epithelial monolayers, macromolecular permeability was measured. Co-culturing IEC with EGC significantly enhanced TER within 48hrs ($p<0.001$). Immediately after TNFα-stimulation, a dose-dependent drop in TER was observed in the absence of EGC, most likely reflecting tight–junctional alterations because there were no detectable TNFα-induced increases in macromolecular permeability at any concentration or time-point. Epithelial integrity was fully preserved when IEC were treated with 10ng/ml TNFα ?in the presence of EGC ($p<0.001$ vs. stimulated IEC w/o EGC). However, EGC could only partially prevent TNFα-effects on epithelial tightness when IEC were exposed to 1 or 100ng/ml TNFα. Forty-eight hrs after TNFα exposure, all TNFα-treated IEC monolayers displayed a marked recovery with a significant rise in TER-values ($p<0.005$). This increase was potentiated if TNFα-stimulated EGC were present in the basolateral compartment ($p<0.001$). Similarly, adding EGC to IEC after TNFα-exposure significantly augmented TER values after at least 48hrs of coculture ($p<0.001$). Adding IL-1β to the coculture system effected less pronounced TER-alterations, whereas both IL-13 and IFNγ induced a fulminant decrease of epithelial tightness which only slowly recovered during the 48hrs observation period. In summary, the intestinal epithelial barrier appears to be supported and enhanced by EGC. While inflammatory mediators like TNFα, IL-1β, IFNγ, and IL-13 diminish the tightness of IEC, most likely via alterations of epithelial tight junctions, these effects are prevented or mitigated in the presence of EGC. These data suggest a protective potential of EGCs for the intestinal epithelial barrier in intestinal inflammation which may depend on the immunological profile of the inflammatory response. Supported by Broad Medical Research Program IBD-0105R.

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Increased proliferation of interstitial cells of Cajal due to altered desensitization of c-kit

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Impaired function of c-Kit, a receptor tyrosine kinase, results in loss of interstitial cells of Cajal (ICC) but little is known about the signaling pathways that c-Kit activates in ICC. We studied transgenic c-Kit gain

of function (Kit GOF) mice with targeted mutations of serine residues 739 and 744 to alanines that are predicted to cause reduced desensitization of c-Kit. These mutations result in increased activation of pathways activated by c-Kit, particularly the phosphatidylinositide 3' kinase pathway. The aims of this study were to determine if Kit-GOF mice developed neoplasms including gastrointestinal stromal tumors (GISTs), determine if ICC were altered in the stomach and to establish the mechanism by which the mutations affect ICC. Kit-GOF mice reproduced and grew normally. No evidence of GISTs were detected in Kit-GOF mice up to 2 years old (n = 10 mice, 12–24 months old; n = 5 mice, 6–8 weeks old). ICC networks in the gastric fundus and body, small intestine and colon appeared normal. However, the density of c-Kit immunoreactivity in the gastric body was higher in the Kit-GOF mice as determined from the 3D reconstructed volumes of confocal images of the ICC (GOF 8.5 ± 0.8, CTL 5.2 ± 0.8% of total muscle volume, n = 4 mice, $p < 0.05$). ICC numbers were counted in the fundus. The numbers of ICC in the fundus were higher in Kit-GOF mice (GOF 10,420 ± 1440, n = 4, CTL 4,920 ± 460 cells/mm^3 tissue, n = 4 mice, $P < 0.05$). The muscle layer was significantly thinner in the fundus of Kit-GOF mice (GOF 54 ± 6 μm n = 4 mice, CTL 89.0 ± 0.7 μm n = 4 mice, $p < 0.02$). Primary cultures enriched in ICC from the small intestine of 4–6 day old Kit-GOF mice contained more dividing ICC as detected by labeling for Ki67 and c-Kit (Kit-GOF 33 ± 2% of ICC positive for Ki67, Control 14.4 ± 4.3 %, n = 3). The Kit S739A/S741A mutation results in larger numbers and volumes of ICC in vivo and more proliferation of ICC in vitro. We conclude that activation of c-Kit supports ICC networks in part by activating proliferation of ICC and that this effect can be enhanced by reducing desensitization of the Kit receptor. Supported by NIH DK52766 and DK57061.

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H_2S mediates smooth muscle relaxation through K channels in human and rat colon

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Hydrogen sulphide (H_2S) is probably an endogenous gaseous neurotransmitter present in the central and peripheral nervous system. In vascular smooth muscle H_2S cause relaxation and hypotension but the role of H_2S in the gastrointestinal tract is unknown. The aim of this study was to investigate the effect of H_2S on the spontaneous motility of human and rat colonic smooth muscle. Muscle bath experiments were performed with circular strips obtained from Sprague Dawley rats (n=6) and surgical specimens obtained for rectal neoplasm (n=5). The amplitude of spontaneous contractions (AUC) was measured before and after drug addition. Cumulative dose-response curves of H_2S, using NaHS as a donor, were calculated in order to estimate the IC_{50}. H_2S dose dependently inhibited the spontaneous motility in human colon IC_{50} 261μM (log IC_{50}-3.58± 0.05) and rat colon IC_{50} 31μM (log IC50-4.5±0.04). No major differences were found when dose response curves were performed in the presence of the neural blocker tetrodotoxin (TTX 1μM): Human: IC_{50} 183μM (log IC_{50} -3.73±0.08 ns) and Rat: 26μM (log IC_{50}-4.57±0.04 ns). These results suggest that H_2S is causing smooth muscle relaxation post-junctionally. In the presence of TTX, TEA (10mM) significantly reduced the inhibitory effect induced by H_2S (Both human and rat: two-way ANOVA $P<0.0001$). The NaHS IC_{50} was shifted to 2421μM (log IC_{50}-2.6±0.06) for human colon and 674μM (log IC_{50}-3.1±0.04) for rat colon. These results suggest an involvement of a K channel in the inhibitory response of H_2S. In the presence of TTX (1μM) the potassium channel blockers glybenclamide (10μM) and apamin (3μM) significantly reduced the inhibitory effect of H_2S. The KATP channel blocker glybenclamide increased the NaHS IC_{50} to 2464μM (log IC_{50}-2.6±0.1 two-way ANOVA $P<0.0001$) for human colon and 80μM (log IC_{50}-4.0±0.04 two-way ANOVA $P<0.0001$) for rat colon. The SK(ca) channel blocker apamin increased the NaHS IC_{50} 1887μM (log IC_{50}-2.77±0.06 two-way ANOVA $P<0.0001$) in human colon and 167μM (log IC_{50}-3.77±0.06 two-way ANOVA $P<0.0001$) in rat colon. We conclude that H_2S inhibits colonic smooth muscle cells through a mechanism involving K^+ channels, possibly ATP sensitive K^+ channels and small conductance calcium activated K^+ channels. In this work we provide functional data showing that H_2S may be considered the third gaseous inhibitory neurotransmitter in the human gastrointestinal tract that should be further investigated.
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Rome II vs. Rome III criteria for functional gastrointestinal disorders in pediatric patients evaluated for abdominal pain

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The pediatric Rome II criteria for functional gastrointestinal disorders have been updated to reflect advances in the literature. This study examined the extent to which application of Rome II versus Rome III criteria would yield different frequencies of FGIDs in pediatric patients with chronic abdominal pain and no evidence of organic disease.

Patients (n = 364) were ages 8–15 years (62% female). The parent-report Questionnaire on Pediatric Gastrointestinal Symptoms (QPGS, Caplan, Walker & Rasquin) was scored according to both Rome II and Rome III criteria.

The majority of patients met criteria for one or more FGIDs (Rome II, 74%; Rome III, 86%). Multiple FGIDs were more frequent with Rome III scoring (24%) than Rome II scoring (16%). Criteria were most frequently met for Irritable Bowel Syndrome (IBS) (Rome II & III, 46%), consistent with previous studies (Caplan et. al, 2005b; Schurman et al., 2005; Walker et al., 2004). The greatest change in frequency was for Abdominal Migraine (Rome II, 6%; Rome III, 22%). The table shows percentages of patients meeting criteria for each FGID included in both Rome II and Rome III classifications (numbers do not total 100% because some patients met criteria for multiple FGIDs).

Application of Rome II and Rome III criteria resulted in similar classification of patients across most FGIDs. Exceptions included a higher frequency of Abdominal Migraine and multiple diagnoses with Rome III. The updated criteria classify more pediatric patients as meeting criteria for one or more FGIDs, suggesting increased utility to clinicians and researchers who identify and treat pediatric FGID.

FGID	Functional Dyspepsia	IBS	Abd. Migraine	Functional Abd Pain	Functional Non-Retentive Fecal Soiling	Aerophagia	Cyclic Vomiting
Rome II	Ulcer-like: 2% Dysmotility-like: 2% Unspecified: 16%	46%	6%	4%	2%	9%	3%
Rome III	15%	46%	22%	8%	1%	9%	3%

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Characterization of the pyloric sphincter using high resolution manometry

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Background: Measurement of antroduodenal pressure is commonly performed and several catheters are available for clinical use. Recording from the pyloric channel is more challenging due to peristalsis and is usually performed with a sleeve device. Recently, a solid-state, high-resolution manometry system was developed which incorporates 36 circumferential pressure sensors spaced at 1-cm intervals and contour plot software. Our aim was to perform APD manometry with this system to determine whether it provided reliable and precise manometric measurements of the pyloric region. **Methods:** Ten healthy subjects (7M:3F) were recruited. After upper endoscopy the catheter (ManoScan^{360}) was introduced transnasally and advanced across the pylorus with the aid of an endoscope. In final position, confirmed fluoroscopically, 15 to 20 sensors were in the stomach and the

remainder distributed across the pylorus and duodenum. Patients were recorded fasting and then given either a liquid nutrient or scrambled egg meal and recorded post-prandially. **Results:** Length of recording was 172.9 ± 21.8 minutes fasting and 61.5 ± 7.6 minutes post-prandially. All subjects demonstrated all three phases of the MMC. The duration of phases I, II and III were 103.6 ± 25.0, 25.9± 6.3, and 10.3 ± 0.9 minutes respectively. Nine subjects converted into the fed pattern after the study meal. Using pressure data and contour plots, the pylorus was identified in all subjects. Mean pyloric width was 2.07 ± 0.13 cm (95% CI; 1.40–2.40). Pyloric resting pressure during phase I was 9.4 ± 1.1 mmHg while antral pressure was significantly lower (p= 0.003; 95% CI; 2.4 - 8.4). Pyloric pressure was always elevated relative to the antral pressure in phase I. For phase II and III, pyloric pressure was 7.7 mmHg ± 2.3 and 9.4 ± 1.1 mmHg respectively. Pyloric pressure increased equally after both the liquid and solid meal. Several subtypes of APD pressure events and waves were recorded including isolated pyloric pressure waves. **Conclusions:** By using a solid-state catheter system to perform APD manometry, we were consistently able to identify the pyloric sphincter and show that it had a pressure approximately 5 mmHg above antral pressure during Phase I and that it increases in the post-prandial state. Isolated pressure events and waves which involve the pylorus were readily identified.

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Enhanced expression of genes associated with visceral hypersensitivity in small intestine of IBS patients

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Background: Hypersensitivity plays an important role in IBS. Many studies have shown increased visceral perception to mechanical distension in the colonic and small intestinal region. IBS patients were found to have an increased serine protease activity in the colonic mucosa. Moreover, IBS mucosal supernatant-induced hyperalgesia in response to rectal distension was abolished after pre-incubation with serine protease inhibitor. Upregulation of trypsinogen IV, expressed by epithelial cells, may be responsible for the increased activity. Trypsin IV, active form of trypsinogen IV, is an agonist of protease-activated receptor (PAR) 2. Increased PAR2 expression and/or activation causes hyperalgesia. Furthermore, alterations in components of serotonergic signalling that affect serotonin (5HT) availability, such as its biosynthesis or uptake may lead to changes in visceral sensitivity. Tryptophan hydroxylase 1 (TPH1) is the rate-limiting enzyme in the 5HT biosynthesis and SERT is the transporter responsible for 5HT uptake. Abnormalities in mucosal serotonergic signalling in rectal biopsies of IBS patients have been identified. **Aim:** Determine the role of trypsin IV, PAR2 mediated effects and altered serotonergic signalling in visceral hypersensitivity of the small intestine in IBS. **Materials and methods:** Duodenal mucosal biopsies of 34 IBS patients and 20 healthy subjects (HS) were collected. According to Rome II criteria the patients could be divided in 10 constipation predominant (IBSC), 11 diarrhea predominant (IBSD), and 13 alternating IBS patients. PAR2, trypsinogen IV, TPH1 and SERT transcripts were quantified using real-time PCR and relative standard curve method. **Results:** IBS patients showed 1.5-fold higher trypsinogen IV mRNA level, normalized against PBGD, compared to HS (P=0.003). IBSD and IBSC patients exhibited 1.8-fold higher trypsinogen IV expression compared to HS (P=0.01, P=0.016). Normalization against GAPDH confirmed these results. SERT expression, normalized against PBGD, was 1.9-fold higher in IBS patients compared to HS (P=0.01). This higher SERT expression could not be attributed to a specific IBS subgroup, although a trend for IBSC (P=0.063) was observed. No statistical differences in TPH1 and PAR2 transcript levels were found irrespective of the housekeeping gene used. **Conclusions:** Trypsinogen IV and SERT expression are higher in IBS patients compared to HS. Higher levels of trypsinogen IV may increase the activation of PAR2. Increased SERT expression may lead to altered 5HT availability. Both may contribute to small intestinal visceral hypersensitivity in IBS patients.

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Is hypersensitivity to drugs an extra GI somatic manifestation of IBS?

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Background: In clinical practice, patients with IBS frequently complain of medication side-effects (and often discontinue treatment), but the prevalence of it has never been evaluated. **Aims:** The first aim of this study was to assess the prevalence of drugs intolerance as an extra GI manifestation in patients with IBS. The secondary aim was to verify the association between drugs intolerance and psychological comorbidity. **Methodology:** Female patients followed in a tertiary care center completed questionnaires assessing the presence of drugs hypersensitivity as well as 11 somatic and 4 psychological extra GI conditions. IBS patients (Rome II criteria; n=71) were compared to inflammatory bowel disease patients (IBD; n=96) or to healthy controls (HC; n=67); 2) The relationship to psychological comorbidity was verified in two different paradigms: a) by looking at the statistical correlation between drugs intolerance and the psychological extra GI symptoms in our IBS patients, and b) by comparing in a meta-analysis the side-effects to placebo (nocebo effect: presumably increased due to hypervigilance or amplification in psychological disorders) in 1305 IBS patients or in 42585 patients with comparable medical conditions included in various drug trials approved by Health Canada (equivalent to FDA) between 1993 and 2003. **Results:** Prevalence of drugs intolerance was significantly more elevated in IBS (41% of patients) than in HC (7%) or in IBD (27%); as expected, biological and psychological extra GI symptoms were also markedly increased in IBS; 2a) In our IBS patients, drugs intolerance was significantly associated with somatic comorbidities such as fatigue ($p < 0.001$; OR 10.5), urinary symptoms ($p = 0.015$; OR 3.8), hypoglycemia (OR 3.5), but not with psychological factors such as depression, anxiety, mood instability, or sleep disorder; 2b) The meta-analysis revealed that nocebo effect was not different in patients with IBS than in patients with other comparable medical conditions. **Conclusions:** Drugs intolerance or hypersensitivity is a frequent extra GI manifestation of IBS, and it was not associated with psychological comorbidity; a somatic origin must therefore be explored.

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Exacerbation of irritable bowel syndrome symptoms during menses is associated with increased prostaglandin (PGE_2) levels

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Background: In IBS patients, pain and diarrhea often increase during menses when estrogen and progesterone levels are lowest. However, IBS symptoms improve after menopause when estrogen and progesterone levels are also low, indicating that estrogen levels are poorly correlated with IBS symptoms. Estrogen and progesterone effects on IBS may be indirect and mediated through the rapid drop in these hormones at menses that indirectly causes a rise in prostaglandins. **Aim:** Determine whether women who report exacerbation of IBS symptoms during menses (IBS-X) have higher levels of prostaglandins (PGE_2, PGE_2 metabolite) in menstrual fluid and in serum, compared to women with IBS not exacerbated during menses (IBS-noX) and to healthy controls (HC). **Procedure:** 17 IBS-X, 22 IBS-noX, and 18 HC were evaluated on their first day of menstrual bleeding for measurement of serum and menstrual fluid levels of PGE_2. Eight ml of venous blood was drawn, then a previously prepared tampon, made from dialysis tubing and containing Dextran, was inserted into the vagina. The subject then rested quietly on her back for 60 minutes while the concentration of PGE_2 from menstrual fluid came into equilibrium with the concentration of PGE_2 in the Dextran within the dialysis tubing. Blood and menstrual fluid were analyzed by radioimmunoassay for levels of PGE_2 and PGE_2 metabolite, which were compared among the three groups. **Results:** Serum PGE_2 was elevated in the IBS-X

group compared to the IBS-noX (p=.008, Mann-Whitney), with a trend favoring IBS-X compared to HC (p=.089, Mann-Whitney). Similar trends were seen in the menstrual fluid levels of PGE_2, and PGE_2 metabolite, but the differences were not statistically significant. The IBS-X group showed higher serum levels of PGE_2 metabolite than the IBS-noX group (p<.001, Mann-Whitney). However, differences between the IBS-X and HC groups were not significant. **Discussion:** IBS patients whose symptoms worsen with menses show elevated PGE_2 in serum and menstrual fluid compared to IBS patients whose symptoms do not worsen with menses and compared to healthy controls. These elevations in the pro-inflammatory mediator, PGE_2, may be responsible for the exacerbation of abdominal pain and loose stools during menses in IBS patients. [Supported by R24 DK67674, RO1 DK31369, RR00046, P30 DK34987].

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Long-term safety and efficacy of lubiprostone for the treatment of chronic idiopathic constipation

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Constipation is a common gastrointestinal condition with often ineffective treatment options. Lubiprostone, a novel type-2 chloride channel activator (*Am J Physiol Cell Physiol.* 2004;287:C1173-C1183), has been shown to be efficacious and well tolerated by patients with chronic constipation in clinical trials of 3 to 4 weeks' duration (*Gastroenterology*. 2002;122:A315, *Gastroenterology*. 2003;124:A38, *Am J Gastroenterol.* 2005;100:S324, S328, S329). We describe results from three open-labeled, long-term (6 to 12 months) trials where assessments were periodically captured (approximately every 6 weeks). Methods: Study 1 was a 24-week, open-labeled extension to a 4-week, double-blind, pivotal trial that enrolled 308 patients. Study 2 was a 48-week, open-labeled trial in 248 patients, 87 of whom were examined in a 7-week randomized withdrawal study period prior to the open-labeled phase of the study. Study 3 was a 48-week open-labeled trial in 324 treatment-naïve patients. Patients assessed treatment effectiveness, constipation severity, and abdominal symptoms of bloating and discomfort using a 5-point scale. Results: Improvements in constipation severity, abdominal bloating, and abdominal discomfort were statistically significant at all visits (p<0.0001). Constipation severity was improved by an average of 1.28 points at Weeks 4 to 6 (N=828), 1.47 points at Week 24 (N=512), 1.38 points at Week 48 (Study 2 and 3 only, N=281), and 1.15 points for the last on-drug measurement (N=866). Abdominal bloating was improved by an average of 0.89 points at Weeks 4 to 6 (N=829), 0.98 points at Week 24 (N=512), 1.00 points at Week 48 (Study 2 and 3 only, N=282), and 0.79 points for the last on-drug measurement (N=867). Abdominal discomfort was improved by an average of 0.74 points at Week 1 (N=619), 0.91 points at Week 24 (N=512), 0.87 points at Week 48 (N=282), and 0.72 points for the last on-drug measurement (N=867). Lubiprostone was well tolerated. The most commonly reported, related adverse events (≥5% of patients) were nausea, diarrhea, headache, abdominal distension, flatulence, and abdominal pain. Conclusion: These results demonstrate that the improvements observed in the short-term, double-blind trials are maintained for at least 24 to 48 weeks, as shown in these three long-term trials. Lubiprostone provides significant relief for a variety of constipation symptoms, and efficacy is sustained for up to 48 weeks. This research was funded by Sucampo Pharmaceuticals, Inc.

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Alternative antiadrenergic pharmacodynamics of BoNT/A

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Chronic Anal Fissure (CAF) is associated with Internal Anal Sphincter (IAS) spasm which produces local ischemia and delays the healing of the lesion. Botulinum neurotoxin A (BoNT/A) is currently used to treat this disease but its precise mechanism of action on anal sphincter dynamics is still under discussion. BoNT/A blocks neuroexocytosis with selectivity for cholinergic fibres. However, acetylcholine is known to cause IAS relaxation, while norepinephrine to mediate contraction: consequently, BoNT/A should produce an increase, not a fall, in IAS tone. Human IAS adrenergic nervous fibres were investigated to identify the synaptosomic protein ''SNAP-25'', the molecular target of BoNT/A. Histologic sections of human IAS were processed for immunofluorescence double labeling reactions, using anti-TH (tyrosine hydroxylase) and anti-SNAP-25 (synaptosomal associated protein of 25 kDa) antibodies. The microscopic analysis showed positive labeling for both immunoglobulins. Confocal laser scanning fluorescence microscopy proved colocalization of SNAP-25 and TH in nervous fibres to be found in connective interstices of the sphincteric smooth muscle cells and in the space that separates the internal sphincter from the longitudinal muscular layer. This neuromorphologic data could explain the therapeutic action of BoNT/A in anal fissure. The presence of SNAP-25 in adrenergic nervous fibres would justify the relaxation effect of BoNT/A on IAS with a norepinephrine releasing inhibition. This is the first published example of BoNT/A therapy mediated by an antiadrenergic effect.

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Late onset of hirschprung's disease: A diagnostic dilemma

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Background and aims: Hirschprung's disease (HD) is almost always a disease within pediatric realm. Adult HD is an infrequently diagnosed entity that must be suspected in a patient with chronic, intractable constipation since infancy; however, it turns into a more rare condition when its symptoms emerge late in adulthood. We report some cases of HD with late onset of symptoms. **Methods:** From September 2004 to February 2006 we encountered 9 cases of adult HD. Among them, 3 patients manifested with late onset of the related symptoms. Demography, clinical manifestations and paraclinical findings of these patients are discussed. **Results:** The 1st case was a 23 year-old female in a good condition until the age of 21 years, when she manifested with an intractable chronic constipation, not alleviated unless with laxative use or enema. She had a history of hypothyroidism and secondary amenorrhea. One year after the beginning of constipation, rectosigmoidoscopy and biopsy was performed for her which confirmed the diagnosis of Crohn's disease. She was treated, for one year period. However, her constipation did not resolve. When she referred to our clinic, we performed barium enema and anorectal manometry for her. Dilatation of upper rectum and sigmoid colon and absence of rectoanal inhibitory reflex were detected. Full thickness rectal biopsy confirmed the diagnosis. The 2nd case was a 17 year-old female, with a history of imperforate anus operated at the day 40 of her age. Her complain of constipation began when she was 13 years old, four years after which she was referred to our clinic. We performed barium enema and anorectal manometry for her. The findings were compatible with HD, which was confirmed by histopathology. The 3rd case was a 24 year-old female, with a history of chronic constipation within the past 4 years. She also suffered from absence type epilepsy. Rectosigmoidoscopy revealed dilatation of upper rectum and narrowing of the lower part. The diagnosis of HD was established with manometry and histopathology. **Conclusion:** In adult patients with long standing refractory constipation since birth, infancy or childhood and in a few cases since adulthood, Hirschprung's disease should be ruled out. Anorectal manometry and barium enema are helpful in this setting. The probable link between HD and other diseases needs to be assessed.

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Magnetic resonance imaging of idiopathic megarectum during distension

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The activation of rectal mechanoceptors as a result of compression or distortion is enhanced by active smooth muscle contraction [J Physiol 2005; 564.2: 589–601]. Rectal sensitivity is severely impaired in patients with idiopathic megarectum, but whether this alteration is mainly related to rectal hypotonicity or the lack of significant rectal wall distortion during distension has not been investigated. Magnetic resonance imaging allows an assessment of the spatial relationship between the distending balloon and rectum. **Methods:** Four female patients with idiopathic megarectum (defined as a rectal diameter of >6.5 cm at lateral X-ray) aged 18–28 years underwent rectal magnetic resonance imaging before and during distension with an 8-cm plastic bag at volumes ranging from 50 to 250 ml in 50-ml steps, and the results were compared with those previously found in nine healthy subjects [Dis Col Rectum 2005;48:1220–7]. Rectal distensibility and sensations during stepwise, barostat-pressure controlled distensions (up to 44 mmHg or intolerance) were also assessed in the same four patients and 17 healthy subjects (7 males, 10 females aged 21–47 years). The data are given as mean values ± SD. **Results:** Before distension, the radius of the rectum of the idiopathic megarectum patients, dilated by intraluminal contents, was 3.1±0.4 cm, whereas the healthy subjects had a nearly collapsed lumen. During balloon distension, the intraluminal contents were displaced cranially or compressed laterally, and there was a significant increase in rectal radius to 4.4±0.1 cm (P=0.01) and anterior compression of the rectovesical space (from 2.8±0.8 cm at 0 ml to 1.8±0.5 cm at 250 ml; P=0.01). The patients reported a desire to defecate at greater volumes than the healthy subjects (230±130 ml *vs* 141±77 ml; P=0.06); urgency was reported by all of the healthy subjects, but none of the patients (P<0.01). Resistance to inflation was less in the patients, whose pressure/volume ratios at 8 mmHg were significantly lower than those of the healthy subjects (0.027±0.0025 *vs* 0.098±0.055; P=0.02). **Conclusions:** Patients with idiopathic megarectum have a hypotonic rectum and blunted rectal sensations despite the distortion of their rectal and perirectal structures during distension. These results suggest that rectal hypotonicity plays a major role in the reduced perception of sensations.

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Biofeedback treatment improves quality of life in patients with fecal incontinence

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Objective: To analyze change in severity of fecal incontinence (FI) and quality of life (QL) in patients with FI after biofeedback treatment. **Methods:** Prospective, non-randomized study in 40 patients (3M/37F; mean age: 58) with FI treated with biofeedback. All patients filled out both a questionnaire on FIQL and the Cleveland scale on FI severity before and after treatment. Changes were analyzed by way of Student t test for paired data, Wilcoxon Signed Rank test and Pearson correlation coefficients. p<0.05. **Results:** FI severity was significantly reduced after treatment (see Table) in all the analyzed parameters (incontinence of solid/liquid stools/gases, and use of anal protectors). A statistically significant increase of the mean values in the 4 domains of the FIQL: lifestyle, behavior, depression/self perception and embarrassment (see Table). The Wilcoxon test showed statistical significance in the changes observed for all the post-biofeedback severity and QL parameters. There was a statistically significant correlation between the post-treatment changes in the different FIQL domains and the changes observed in the FI severity score (quality of life, r −0.55, p<0.001; behavior, r −0.55, p<0.001; depression, r −0.4, p<0.01; embarrassment, r −0.39, p<0.01). **Conclusions:** Treatment of FI patients with biofeedback significantly improves their quality of life and reduces the severity of fecal incontinence.

Table 1 Pre- and post-treatment means and mean differences (Student t paired data)

	Basal (pre-treatment)	Postbiofeedback	
	Mean (SD)	Mean (SD)	Mean difference (SD)
Cleveland score	15.3±4.5	9.7±6.2	5.6±5*
Solid stool inc	2.6±1.5	1.3±1,5	1.3±1.4*
Liquid incontinence	3±1.3	1,5±1.7	1.5±1.5*
Gas incontinence	3.5±0.9	2.6±1.6	0.9±1.4*
Use of Pad	3±1.7	2.5±1.9	0.5±1.5*
FIQL: Lifestyle	2.4± 1	3±0.9	0.6±0.8*
Behavior	1.9±1	2.7±0.9	0.7±0.7*
Depression	2.7±1	3.1±1	0.5±0.9*
Embarrassment	2.2± 1	2.8±0.9	0.7±0.9*

*p < 0.001

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The frequency of the sampling response does not determine urge to defecate in patients with constipation: A study using semi-ambulatory anorectal physiology

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Introduction: The sampling response describes reflex lowering of anal sphincter pressure allowing rectal content to come into contact with sensitive mucosa in the anal canal. Sampling events allow discrimination of faeces from flatus and may contribute to the perception of a rectal urge to defecate. Constipated patients can be classified according to whether they have a normal rectal urge to defecate (NUD) or reduced urge to defecate (RUD)[1]. Infrequent sampling events may cause RUD. Semi-ambulatory simultaneous anal and rectal manometry was used to record sampling events. **Method:** Prospective study comparing frequency of sampling events in NUD patients with RUD patients. Ethical approval obtained. Power calculation to determine sample-size based on 4-hours of recording. Subjects with functional constipation (Rome II criteria) recruited. Colonic transit measured with radio-opaque markers. Resting anal pressure (RAP), anal squeeze pressure (ASP), rectal pressure (RP) and anal electromucosal sensation recorded. Rectal balloon distention to assess RAIR and rectal sensation (barostat controlled inflation). Semi-ambulatory anorectal manometry using a catheter with 2 solid-state transducers at 1cm and 5cm (Gaeltec). Catheter attached at anal verge with the transducers in the rectum and anal canal. The subject was ambulatory within the department during recording. Recordings reported by a researcher blinded to whether the subject had NUD or RUD. **Results:** 22 female patients studied; 12 RUD;10 NUD. No differences between groups re: age; duration and severity of symptoms or mean duration of recording - NUD group 3.95 hrs, RUD 3.86 hrs (NS). Mean sampling event frequency: NUD group 8.71/hr (range 2–20/hr), RUD 8.95/hr (range 0–21/hr) (NS). NUD patients perceived 6% of sampling events; RUD patients perceived 9% of events (NS). No difference in total or segmental colonic transit time between groups. No association between sampling event frequency and segmental or total colonic transit time. No difference in RAP, ASP, RP or rectal and anal sensation between groups. **Conclusion:** The cause of RUD in these patients was not infrequent sampling events. The factors determining whether patients have RUD or NUD remain unclear. Further study is required to evaluate rectal compliance, rectosigmoid motility and higher cerebral function in determining urge to defecate.

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Validity of segmental transit studies in identifying obstructed defecation in patients with functional constipation

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Introduction: Segmental colonic transit can be determined by radio-opaque markers studies. The Rome III criteria[1] for functional constipation (FC) state: ''*retention of markers in the proximal or transverse colon suggests colonic dysfunction, and retention in the recto-sigmoid area suggests obstructed defecation*''. **Aim:** To determine whether predominant retention of markers in the rectosigmoid characterizes the type of constipation, by examining transit study results from patients classified into Obstructive Defecation (OD) and Non-Obstructive Defecation (NOD) by symptom and proctographic parameters. **Method:** Consecutive FC patients (Rome II criteria) assessed by radio-opaque marker study[2] and radio-isotope defecating proctography[3]. OD classification by proctography using recognized parameters; Evacuation rate, Evacuation time, Pelvic Floor Descent, % Evacuation[3]. OD classification by Likert scoring of obstructive symptoms: straining, incomplete evacuation, digitation, cumulative OD score. Transit results included total-transit-time in hours (T-total); rectosigmoid transit time (T-RS), geometric marker distribution (GMD). Analysis using t-test, Pearson correlation & kappa coefficient. **Results:** 108 patients (6% male), median age 41 yrs. Complete data for all patients. Transit results for OD & NOD classified by proctographic parameters: No differences between groups (p NS). Transit results for OD & NOD classified by straining, digitation, OD score: No differences between groups (p NS). Classified by incomplete evacuation: T-total NOD (59 hrs) and T-total OD (52hrs) (p=0.03). Kappa coefficient showed poor agreement between symptom & proctography parameters for classifying as OD. **Conclusion:** These data do not support the hypothesis that segmental transit assessment characterizes the type of constipation. Patients exhibiting OD according to symptoms or proctography are no more likely to have recto-sigmoid retention than those with NOD. The validity of segmental transit estimation remains dubious. In addition, the value of classifying OD by symptoms is questionable due to poor agreement with proctographic parameters.

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Does successful sacral neuromodulation alter rectal sensory function and compliance in patients with faecal incontinence?

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Background: Sacral nerve stimulation (SNS) may improve faecal incontinence (FI), but its mechanism of action is poorly understood. Rectal sensation and wall compliance are known to be important in maintaining continence. We aimed to investigate whether these were altered in response to successful temporary and permanent SNS. **Methods:** Eleven patients (all female; median age 52 [range 26–75]) with refractory FI underwent successful temporary SNS, 9 of whom subsequently underwent permanent implant. Comprehensive anorectal physiological assessment was performed, including rectal sensory testing to both simple volumetric (latex balloon) and pressure-based barostat distension, and measurement of compliance. This was done pre-stimulation, after 14 days of temporary, and after 1 month of permanent stimulation. Sensory thresholds for desire to defecate (volume; DDV and pressure; DDP), and maximum toleration MTV; MTP) were recorded, and compared with those obtained in healthy female volunteers (balloon: n=27; barostat: n = 18). **Results:** As a group, successful temporary or permanent stimulation were not associated with any significant changes in simple volumetric or pressure-based barostat sensory thresholds, or rectal compliance. However, analysis of individual data revealed that: 1) of 4 individuals, hypersensate to volumetric distension at baseline (MTV <90 mls), 3 became normosensate with SNS; 2) 1 individual became hypersensate following stimulation; 3) of 4 individuals hypocompliant prior to SNS, all remained so; 3 / 4 were hypersensate at baseline, and the fourth was the individual who became hypersensate following SNS; 4) 1 individual, hyposensate prior to treatment (MTV >360 mls), remained so. For pressure thresholds: 1) no patient had an abnormal DDP at baseline, or following SNS; 2) 2 patients with normal MTPs had abnormally elevated thresholds (hyposensitivity) after intervention. **Conclusion:** Despite subjective clinical improvements following SNS, this intervention has no consistent effect on rectal sensorimotor behaviour. Nevertheless, assessment of individual data reveals normalisation of heightened sensation on simple volumetric testing in the majority, independent of compliance. However, a similar effect was not reflected using pressure-based thresholds. Lack of consistency may be due, in part, to the pathophysiological heterogeneity of the study population, or inadequate methodology in relation to the pathophysiology. This study highlights the importance of analysis of individual, as well as group data.

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Elevated rectal mucosal substance P levels in patients with faecal incontinence are normalised by successful sacral nerve stimulation

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Introduction: Sacral nerve stimulation (SNS) may improve faecal incontinence (FI). The mechanisms for this are unclear but may involve modulation of rectal sensation. Changes in peripheral expression of relevant neuropeptides and receptors in response to SNS have not been specifically sought. This was the aim of this study. **Methods:** Eight endoscopic rectal mucosal biopsies were taken from each of 12 patients before (baseline) and after 14 days of temporary SNS, and also at 90 days after permanent stimulation from 10 responders (reduction of weekly episodes of FI by >50%). Multiple tissue sections from each biopsy were immunostained for substance P, TRPV1, VIP, CGRP and neural markers (neurofilament / peripherin). Blinded quantification of staining (% area immunoreactivity) was performed with computer-assisted image analysis (Olympus DPSoft) with baseline levels also compared with identical biopsies from 8 asymptomatic volunteers. **Results:** Baseline SP (0.52 [mean] +/− 0.09 [SD] vs. 0.16 +/− 0.04%; $P = 0.001$) and TRPV1 (0.76 +/− 0.35 vs. 0.09 +/− 0.05; $P = 0.0001$) but not VIP (1.26 +/− 0.89 vs. 1.28 +/− 0.89, $P = 0.94$) % area immunoreactivities were significantly increased when compared with controls at baseline. SNS resulted in a significant decrease in SP % area immunostaining after 14 days of temporary (0.21 +/− 0.07) and 90 days of permanent stimulation (0.18 +/− 0.09), P=0.049, two-way ANOVA). There was no change in immunoreactivity in the 2 non-responders at 14 days. No qualitative or quantitative differences in the immunoreactivity of TRPV1, VIP, CGRP or neural markers were demonstrated with temporary or permanent stimulation. **Conclusion:** In patients with FI, elevated rectal mucosal SP normalizes to levels observed in asymptomatic controls with SNS. This appears to be a specific finding in comparison with other neurochemistry. The putative role of SP in smooth muscle contractility, afferent sensation and local vascularity and secretions highlights the relevance of this finding, which warrants further investigation.

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Influence of gender and age on the results of anorectal manometry (ARM)

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Introduction: In the literature, data on the effects of gender and age on the results of ARM differ. Possible reasons are investigation of only small numbers of healthy people and comparison of only two groups with huge age differences. Especially sparse are gender and age data about the sensory parameters. **Aims & Methods:** Aim of the present study was to investigate the influence of gender and age in a huge healthy cohort. ARM was performed in 72 women (W) and 74 men (M) with median ages of 64 years (range: W 22-90yrs; M 23-88yrs). Exclusion criteria were fecal incontinence, constipation, colorectal or neurological diseases and diabetes. We determined mean anal resting (MRP;mmHg) and squeeze pressure (MSP) as well as minimal rectal balloon volume for perception (BVP;ml) and for urge/desire for defecation (BVU). T-test was used to analyse for gender differences, regression analysis to search for age influences. **Results:** MSP (p=0.007) and BVP (p<0.001) are lower in females, while MRP and BVU are similar in females and males. MRP (W p<0.0001; M p=0.03) and MSP decrease (W p<0.0001; M p=0.004) with age. An age-related increase in sensory thresholds is only seen in females (BVP p=0.01; BVU p=0.04). **Conclusion:** Most of the parameters measured by ARM are influenced by gender and age. Therefore, the results of ARM must be interpretated in relation to sex- and age-adapted normal values.

median	women	men	<50yrs W/M	-60yrs W/M	-70yrs W/M	-80yrs W/M	>80yrs W/M
MRP	64	67	81/85	79/87	73/72	52/65	46/68
MSP	151	201	174/265	157/228	144/217	131/204	136/173
BVP	35	40	20/30	15/30	38/30	30/40	30/35
BVU	90	120	73/85	80/128	88/120	83/130	100/130

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Detailed morphological assessment of the anal sphincter complex using the 3-dimensional ultrasound volume and image slice technique

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Background & Aims: We assessed the morphological features of the anal sphincter complex consisting of external anal sphincter (EAS), internal anal sphincter (IAS) and puborectalis muscle (PRM) using 1 mm slices of a 3 dimensional ultrasound (3D US) volume captured by a Phillips HD 11 system. **Methods:** 15 asymptomatic 15 nulliparous women (33±12 years) and 11 asymptomatic multiparous women (49±9 years) were studied. A 3D US volume of the pelvic floor was obtained during rest and squeeze by placing a 3–9 MHz transducer on the vaginal introitus with image directed posteriorly (tranvaginal) and on the perineum with the image directed cranially (transperineal). From the 3D US volume, 2D US images were viewed at every 1 mm distances, in the two volumes to assess IAS, EAS and PRM. **Results:** The 2D US images in the transverse axis of the anal canal (seen in the transvaginal volume) allowed visualization of the IAS and EAS. The entire length of the PRM sling was seen only in the transverse plane of the PRM axis (a line connecting the lower end of the pubic symphysis and the apex of the anorectal angle) in the transperineal volume. A part of the EAS is located below the IAS (subcutaneous portion) and a part around the IAS (superficial portion). The anterior wall of the IAS is longer than the posterior wall. The number of transverse image at 1 mm distance allowed determination of the length of IAS and EAS. The anterior-posterior (AP) length of the PRM was assessed at rest and squeeze. EAS and IAS were similar in the nullipara and multipara women but 2 multipara women revealed breaks in the PRM muscle. The AP length of PRM is longer in the multiparous but both groups show significant shortening with the squeeze. **Conclusion:** Examination of the closely spaced multiple slices of the 3D US volume of the pelvic floor allows detailed morphological assessment of the anal sphincter complex and has the potential to detect damage to its individual components.

	IAS	EAS	PRM AP length (rest)	PRM AP length (squeeze)
Nulliparous (cm)	2.7±0.4	2.1±0.4	5.2±0.5	4.6±0.4*
Multiparous (cm)	2.9±0.4	2.3±0.5	5.7±0.5	4.9±1.3*

*P < 0.001.

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Sacral nerve stimulation in faecal incontinence: are there factors associated with success?

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Background: Sacral nerve stimulation has been used successfully in treating faecal incontinence. Our aims were: 1/ to evaluate the proportion of patients with unsuccessful implantation despite positive test stimulation 2/ to examine and compare factors associated with the success of the transitory and permanent sacral nerve stimulation. **Methods:** A total of 61 patients (55 women) (median age 56 years old, range 33–77) with refractory faecal incontinence, underwent temporary stimulation. A 50% or greater improvement in the number of episodes of faecal incontinence or urgency was required to proceed to permanent implantation and was the criteria of success of permanent sacral nerve stimulation at the last follow-up visit in implanted patients. The factors compared between the success and the failure groups during temporary and permanent stimulation, were patients' age and sex, diagnosis and characteristics of faecal incontinence, previous surgery, quality of life scores, anorectal manometry, endoanal ultrasound, electrophysiological tests performed before stimulation. **Results:** Temporary stimulation was successful in 35 patients (57.4%). A permanent neurostimulation device was implanted in 33. Age was the only factor related to success of the temporary stimulation (p=0.03). After permanent implantation, 31% of patients did not attain screening phase results for the number of episodes of faecal incontinence or urgency. A neurological disorder was more frequently the origin of faecal incontinence in the success group compared to others (p=0.03). The left bulbo-cavernosus reflex was more frequently delayed in the success group than in the others (p=0.03) and a prolonged or absent bulbo-cavernosus reflex was more frequent in the success group than in the failure group (p=0.03). **Conclusion:** Patients with faecal incontinence from neurological origins could be good candidates for sacral nerve stimulation.

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Anatomically based computational models of the male and female pelvic floor and anal canal

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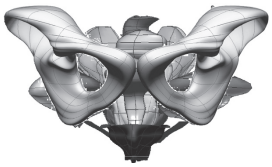
Aim: To provide a framework for examining the functional behavior of the pelvic floor and anal canal. **Background:** Defecation disorders such as fecal incontinence and obstructed defecation are highly prevalent diseases which severely affect quality of life for individuals. We use anatomically-based modeling techniques (integrating both anatomy and physiology) to examine the important mechanisms and muscular components involved in maintaining continence. **Methods:** Realistic three-dimensional anatomically based mathematical models of both the male and female pelvic floor and anal canal regions were constructed using cross-sectional two-dimensional photographs from the Visible Human Project (VHP). This process was then repeated using MRI data obtained from volunteers to create patient specific models. Each structure within our region of interest was manually segmented from the axial slices of the Visible Man and Visible Woman data sets. Each of the segmented data sets was then cross checked against the corresponding coronal and sagittal images. Computational meshes

with derivative continuous boundaries were constructed from the data points using an iterative fitting procedure resulting in meshes with an average RMS error of less than 2 mm (Fig 1). **Results:**



	Rectum		Bladder
	Coccyx		Uterus
	Pubis		Vagina
	Obturator Internus		Transverse Perineae
	Levator Ani		Bulbospongiosus

Fig 1. Visible Woman Model. (Additional pelvic floor structures are hidden from view).

Discussion: The VHP provides the highest quality data set currently available from which to construct these models, however, the images were obtained from cadavers, and thus there is no muscle tone. For this reason, we have repeated this process using MRI studies from normal volunteers for comparison with the existing models.

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Circadian variation of rectal sensitivity is independent of the release of gastrointestinal peptides

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Introduction: An increased awareness of intestinal distention stimuli is typical of the irritable bowel syndrome. Patients often report that their feeling of discomfort varies depending on the time of day. It was the aim of our study to identify possible circadian rhythms for the perception of rectal distention as well as pain stimuli and to correlate them with the release of gastroenteropancreatic hormones. **Methods:** Twelve healthy male volunteers (25.3±0.7 years) were examined. A rectal balloon distention was performed on them. Pressure and volume were double-measured with a computer-controlled barostat (DAISY, Standard Instruments, Karlsruhe, Germany) at the following times: 5.30 p.m. 8.30 p.m., 11.30 p.m., 5.30 a.m., 8.30 a.m., 10.30 a.m. and 1.30 p.m. Subjects were fasted for 6 hours prior to test begin. Around 0.45 a.m. and 10 a.m. they received a standardized meal. Rectal perception thresholds (minimal, urge, pain) were determined and the compliance (pressure/volume relationship) at these thresholds was measured. Thirty minutes before and after each rectal distention a blood sample was taken and the levels of cholecystokinin (CCK), pancreatic peptide (PP) and motilin were determined. **Results:** Sensory thresholds for urge and pain varied significantly on the time of day. With respect to pressure and volume the threshold levels are higher in the evening (9 p.m.) than in the morning (6 a.m.). Bowel wall elasticity showed as well significant differences for urge and pain thresholds. Compliance was plainly higher during daytime than during the evening or night. This difference was significant for the pain threshold. In contrast to motilin, release of CCK ($p<0.001$) and PP ($p<0.002$) showed a significant variation depending on day time. Perception of rectal distention stimuli as well as compliance was independent of intake of food and peptide hormone levels. **Conclusions:** There were significant differences in the perception of rectal distention stimuli for urge and pain depending on daytime. These differences cannot be explained by the release of gastrointestinal peptides. If these circadian variation is learned or conditioned by other factors has to be examined in further studies.

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A study of cerebral blood flow in patients with faecal incontinence secondary to irritable bowel syndrome

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Introduction: It has become clear in our practice that a proportion of the patients complaining of faecal incontinence have irritable bowel syndrome and rectal hypersensitivity. Patients with IBS have different patterns of cerebral blood flow in response to painful colonic stimulation when compared to control subjects. Abnormalities of resting cerebral blood flow have also been recognised in affective disorders such as depression, of which there is an increased incidence, in patients suffering from IBS. We hypothesised that patients with faecal incontinence and irritable bowel syndrome, would have higher psychiatric co-morbidity than controls and that this would be reflected in abnormal resting cerebral perfusion. **Methods:** Patients with faecal incontinence, rectal hypersensitivity and irritable bowel syndrome were recruited from the colorectal clinic at Glasgow Royal Infirmary. Both patients and controls completed the Hospital Anxiety Depression (HADS) score and The Illness Behaviour Questionnaire (IBQ). Patients and controls then underwent a **S**ingle **P**hoton **E**mission **C**omputed **T**omography scan of the brain. Images were quantitatively analysed using statistical parametric mapping (SPM2). The questionnaires were analysed and statistical comparison performed. **Results:** 7 (mean age 48 (9.17)) patients and 10 (mean age 38 (9.85)) controls were recruited. The median HADS-anxiety score for patients was 10 (7–16) compared to 3 (1–8) in controls ($p=0.02$). The median HADS-depression score was higher in patients (6(1–10)) than controls (1(0–6))($p=0.26$). Using the IBQ, patients scored significantly higher than controls with respect to general hypochondriasis (median 1 (range 0–3) vs. 0 (0), $p=0.009$), disease conviction (3(0–5) vs. 0(0–1), $p=0.002$), affective disturbance (3(0–5) vs. 0(0–1), $p=0.003$), irritability (2(1–3) vs. 0(0–3), $p=0.012$) and the Whiteley Hypochondriasis Score (2(0–7) vs. 0(0–1), $p=0.008$). No significant difference in resting regional cerebral blood flow was detected between the control and patient group. **Conclusion:** Patients with irritable bowel syndrome have increased psychological co-morbidity when compared to normal controls. They have higher levels of anxiety and certain aspects of illness behaviour, namely hypochondriasis, disease conviction, affective disturbance and irritability. These relationships are not yet fully understood but may point to a common pathophysiology. These differences in psychological comorbidity in IBS patients do not appear to be reflected in any difference in resting cerebral blood flow when quantified with SPECT scanning.

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Correlation between clinical scoring and sphincter damage in patients with fecal incontinence and normal PNTML

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Background: Two major mechanisms of fecal incontinence (FI) exist – neuropathic & myopathic damage. The relative contribution of sphincter damage to FI among other pathophysiological parameters is unknown. **Aim:** To assess correlation between Wexner clinical scoring system for fecal incontinence in patients with sphincter damage and without pudendal neuropathy **Methods:** Consecutive patients referred for investigation of FI were assessed. All underwent anorectal manometry, endoanl ultrasound (EAUS), pudendal nerve terminal motor latencies and videoproctography in assessing the exact mechanism of FI. EAUS images of the anal canal (AC) were taken at 4 levels – puborectalis, deep, superficial, subcutaneous. For simplicity, AC was considered as symmetrical cylinder. Calculation of the amount of damage was taken as percentage of the entire circumference involved for internal (IAS) and external (EAS) anal sphincters on each of the 4 segments. Total damage for IAS & EAS were calculated as well. **Results:** Of 305 consecutive patients 150 were found to have normal PNTML. 46.7% female. 37 had clinical parameters of constipation and 38 had urinary incontinence as well. Diarrhea was described by 12 patients. 61 (40.7%) were able to hold feces and 31 (22.1%) had insensible fecal loss. Mean defecation frequency was 12/week. Mean Wexner score 9/20. Mean total EAS defect size was 5%. Mean total IAS defect size was 8.7%. Correlation analysis revealed a positive correlation between superficial EAS defect and Wexner score ($r=0.292$,

p=0.02). Resting and squeeze anal pressures are both negatively correlated with Wexner score (r= −0.205, p=0.027; r= −0.222, p=0.0017 respectively).**Conclusion:** The size of sphincter defect is positively related to the clinical severity of FI. Manometry results reflect this parameters.

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Quality of life and psychopathology among patients with chronic idiopathic constipation: a comparison between men and women

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Aim: To explore gender differences in CIC (Chronic Idiopathic Constipation) behavioral patterns and investigate the effects of these differences on mental condition and life quality. **Methods:** One hundred and ninety two patients subjects aged 18–81 (mean 33.4±14.3) participated in the study. 149 females, all fulfilling Rome II criteria for CIC. All patients filled SCL-90 and SF-36 questionnaires in assessing psychopathology and life quality. **Results:** Mean constipation duration 12.6 ±11.9y. Mean defecation frequency 3.2±4.2/w. 20% had less than one defecation per week, 44% 1–2 defections. Twenty percents reported of digitations and 86% patients described straining at evacuation. 53% informed having bloating. 36% didn't have an urge to defecate. More men (95.35%) complained of straining at defecation, as compared to 82.88% of women (p=0.028). There was a tendency (p=0.057) in women (57%) to report of bloating as compared to men (40%). 77% reported using laxatives, significantly more women than men (p=0.0001). Other clinical parameters showed no significant differences. No significant differences were discovered in life quality and psychopathology between men and women. **Discussion:** In this cohort of constipated patients, no significant differences were found in psychopathology and life quality measures. However, women tend to rely on external support (use of laxatives) whereas men use their bodily resources to solve constipation (straining at defecation). Based on these results we suggest that women and men have different attitudes to their illness.

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Fecal incontinence following obstetrical anal sphincter laceration is associated with frequent loose stools

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Background: In the Childbirth and Pelvic Floor Symptoms (CAPS) study, post-partum fecal incontinence (FI) was twice as likely in women with third or fourth degree sphincter lacerations compared to women who delivered vaginally without a recognized sphincter laceration. In other studies, diarrhea was shown to be a risk factor for FI, and antidiarrheal drugs were reported to reduce the severity of diarrhea-associated FI **Aims:** To determine (1) whether chronic diarrhea is associated with an increased risk of FI following sphincter laceration and (2) whether chronic constipation reduces this risk. **Methods:** Women from the CAPS study with 3rd or 4th degree sphincter lacerations who reported urgency to defecate or accidental loss of flatus, solid or liquid stool, or mucus at 6 weeks (n=259) and 6 months (n=218) were included in this study. Diarrhea was assessed by asking women whether their stools were loose or watery 0%, 25%, 50%, 75%, or 100% of the time, and chronic diarrhea was defined by a report of loose or watery stools 75% or 100% of the time. Women were also asked how often their stools were hard or like pebbles, and chronic constipation was defined by reports of 75% or 100%. Accidental loss of solid or liquid stool or mucus, but not flatus alone, defined FI. However, the severity of FI was assessed using the Fecal Incontinence Severity Index (FISI) which includes incontinence for gas. **Results:** Average age of participants was 28 years at 6 weeks. At 6 weeks, 60% of patients with chronic diarrhea had FI compared to 34.8% of those without chronic diarrhea (OR=2.81; CI, 1.96, 3.66). At 6 months, there were similar trends but they were nonsignificant: 36.8% vs. 25.4%; OR=1.72 (CI, 0.73, 2.70). The frequency of loose or watery stools was significantly correlated with the FISI severity score both at 6 weeks (r=0.28; p=0.0002) and 6 months (r=0.14; p=0.03). Hard stools were not protective (contrary to prediction), and in fact tended to be associated with an increased risk for FI at 6 weeks: 56.7% vs. 34.5%; OR=1.66 (CI, 1.07, 2.25). **Conclusions:** The risk of FI following obstetrical laceration is 2 times greater if women have frequent loose stools. Recognizing this added risk factor and individualizing the bowel regimen to keep the stool soft but prevent diarrhea may reduce the incidence and severity of FI associated with sphincter laceration. Supported by grants from NICHD (U01 HD41249, U10 HD41268, U10 HD41248, U10 HD41250, U10 HD41261, U10 HD41263, U10 HD41269, and U10 HD41267).

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Abstract withdrawn

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Subliminal esophageal acid stimulation sensitizes the cortical swallowing network in healthy individuals but not GERD patients

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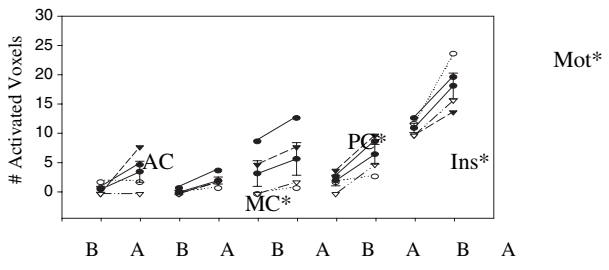
Introduction: Earlier whole-brain studies in healthy individuals have shown increased swallow-related fMRI cortical activity in response to subliminal esophageal acid stimulation. **Aims:** To determine 1. the effect of subliminal esophageal acid stimulation on defined swallowing cortical network in healthy subjects, and 2. compare the findings with GERD patients. **Methods:** 8 right-handed subjects (4 healthy controls, 4 asymptomatic GERD on PPI(1 Barrett's)) were studies by high-resolution functional MRI scanning of the right cortical hemisphere during swallowing (event-related design, voxel size: 1.8x1.8x4.5mm) before(B) and after(A) alternating saline and 0.1 N HCl mid-esophageal infusion at 1mL/min, for a total of 16mL of acid over 42 minutes. We studied the cingulate gyrus, insula, and motor cortex. **Results:** In healthy controls, but not GERD patients, esophageal acid infusion resulted in a significant increase in the number of activated fMRI voxels in the cingulate (middle(MC) and posterior(PC), but not anterior(AC)), insula(Ins), and motor cortex(Mot). (Graph. *p<0.05). None of the subjects experienced heartburn. **Conclusions:** 1. Subliminal acid-induced esophageal sensory input sensitizes the cortical swallowing network in healthy individuals. 2. This effect is absent in GERD patients and may reflect desensitization of the afferent pathways in this group.



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Vagal and non vagal mediated brain activation during gastric distension in pigs

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The central networks involved in the processing of spinal *versus* vagal information originating from gastric distension are poorly understood. Functional imaging of the pig brain before and after vagotomy might give some insights since in pig (i) the brain vascularisation is identical to humans (Vodicka *et al.*, 2000) and (ii) vagotomy is well tolerated without drainage. The aim of this study was to evaluate the difference in brain activation in normal and vagotomized pigs before and during proximal gastric distension.

Brain activation was evaluated using SPECT imaging after IV injection of ^{99m}Tc-HMPAO in 6 pigs (30 ± 5 kg). After insertion of the barostat bag through a permanent stoma, the bag was inflated so to reach 10 or 20 mmHg for 60 s. This procedure was repeated before and 2 weeks after post diaphragmatic vagotomy. Once the desired gastric pressure was reached, the radiotracer was injected IV and by virtue of its chemistry, its brain distribution provides a snap shot of brain perfusion (Warwick, 2004). Retroprojection of brain images were normalized against a SPECT pig template build using 16 animals. Once normalized, the data were analyzed using statistical parameter mapping adapted to the characteristics of the pig brain. The activated region were determined using co-registered MRI atlas (Watanabe *et al.*, 2001). A difference in vagal related brain areas was found before and after vagotomy for low and high-pressure distensions e.g. thalamus, periaqueductal grey, brainstem, olfactory bulb. However, with the exception of cortical activation (mainly frontal), there was no other area with differences in activation. In intact animals, right cortical activations were typically found during gastric distension whereas, controlatereal cortical activations were found during distension in vagotomized animals. Unlike in intact animals, proximal gastric distension after vagotomy was unable to activate the globus pallidus and the hippocampus/amygdala.

Proximal gastric distension was able to activate computational networks (cortical) some of which related to affective functions (Mayberg *et al.*, 1999) while the vagal pathways were severed. Furthermore, there was a right to left shift in cortical activation in vagotomized distended animals. Areas related to process reward (McClure *et al.*, 2004; Singer *et al.*, 2004) were activated only by vagal afferent information. This demonstrates that there is a distinct final network by which vagal versus spinal gastric afferents are processed in the brain.

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Regional brain activation during proximal gastric distension in pigs

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Previous brain imaging study was not able to show a functional neuroanatomic divergence in the processing of noxious and innocuous gastric stimuli. (Vandenberghe *et al.*, 2005). This could relates to the relatively invasive procedure associated with the esophageal introduction of the barostat bag that might cause emotional distress and strong vagal activation as suggested by the author's themselves. Animal models surgically prepared to insert a barostat bag by a stoma might overcome this pitfall. The aim of our study was to evaluate regional brain activation in pigs, an animal model that exhibit brain vascularisation identical to humans (Vodicka *et al.*, 2000).

Brain activation was evaluated using SPECT imaging after IV injection of ^{99m}Tc-HMPAO in 6 pigs (30 ± 5 kg). Briefly, 30 minutes after insertion of the barostat bag through a permanent stoma, the bag was inflated to 0, 10 (no change in food intake) or 20 mmHg (reduced food intake). After 60 sec distension, the radiotracer was injected. Following radiopharmaceutical entrance in neuronal tissue, it undergoes an interaction with the tripeptide glutathione resulting in its inability to wash out neuronal tissue (Warwick, 2004). Retroprojection of brain images were normalized against a SPECT pig template build in the lab using 16 animals. Once normalized, the data were analyzed using statistical parameter mapping adapted to the characteristics of the pig brain. Data were presented as z scores with $p<0.05$ and the activated region were determined using co-registered MRI atlas (Watanabe *et al.*, 2001).

The main regions located along vagal related ascending pathways were activated for the largest distending pressure: brainstem (z=2.22), periaqueductal grey (z=2.22), thalamus (z=2.20) and olfactory bulb (z=3.34). The medulla (z=2.63) and cerebellum (z=2.34) were activated irrespective of the distending pressure. Surprisingly, the globus pallidus (z=2.88) and the hippocampus/amygdala (z=2.08) were also activated for the largest distending pressure.

Unlike the former study performed in humans using PET, brain SPECT imaging was able to identify the activation of the ascending brain vagal pathways. Furthermore, areas related to expected and immediate rewards (McClure *et al.*, 2004; Singer *et al.*, 2004) were also involved in the brain response suggesting that the process reward information network might be involved during gastric distension.

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Recognizing abnormal patterns on PET brain images in adults patients with the cyclic vomiting syndrome

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Background/Aim: Cyclic vomiting syndrome (CVS) is defined as the sudden onset of episodes of intractable nausea, vomiting and abdominal pain with symptom free intervals. Our goal was to investigate whether positron emission tomography (PET) brain imaging can identify abnormal patterns in CVS patients during symptomatic episodes. 8 patients (6 males) meeting Rome II criteria for CVS were studied. 20 minutes after being placed in an environment controlled for light and sound patients were injected with 10 microcuries of Flouoro-Deoxy Glucose and brain metabolic activity data acquired in the 2D mode using a Discovery ST PET/CT Scanner. Quantitative Data from 24 separate areas of the brain were displayed as 47 anatomical regions and compared to a standardized data base which was derived from the PET Brain Scans of 50 normal subjects (Neuro Q). Data are reported as ± SD from the mean. 24 areas of abnormal uptake (hypoperfusion) were identified among the 8 patients. The main areas were right inferior posterior temporal cortex, right/left posterior cingulate cortex, the right anterior cingulate, left inferior frontal. 6 of the 8 patients had decreased activity in the right inferolateral posterior temporal cortex (−2.43), 4 in right posterior cingulate (−2.17), 4 in right anterior cingulate (−1.88), 3 in inferior frontal (−2.68), 3 in left posterior cingulate (−2.45). **Conclusions:** The data from this pilot study of PET brain imaging in adult CVS patients suggest abnormalities in the areas of visual perception, heightened awareness and anxiety as well as pain and pleasure and provide a basis for further studies in this entity.

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Sex-dependent autonomic brain networks in irritable bowel syndrome (IBS)

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Objectives: To characterize the brain networks related to sympathetic nervous system (SNS) activity during rectal balloon distension in patients with irritable bowel syndrome (IBS), and to determine the influence of sex on these networks. **Methods:** Rome I positive IBS

patients (17 females, 17 males) received $H_2{}^{15}O$-PET scans during a resting baseline, rectal balloon distension, and anticipation of distension conditions. Skin conductance (SC) was continuously recorded as a peripheral sympathetic marker and average SC levels for each period were used in the analysis. Multivariate partial least squares (seed PLS) was used to used to identify distributed patterns of activity that were functionally connected with skin conductance across sex and conditions. **Results**: Seed PLS revealed two significant SNS networks: one functioning similarly across conditions and sex ($p<.001$, 39% variance), and another that evidenced sex-specific functioning across the conditions ($p<.01$, 30% variance). The common network included bilateral insula and hippocampus, right (R) amygdala, R ventral lateral prefrontal cortex (vlPFC) [BA 11], left (L) rostral anterior cingulate cortex, L mid and L posteriorcingulate cortex, and L orbital frontal cortex [BA 10]. The sex-specific autonomic network included bilateral thalamus, somatosensory regions [BA 2/1/3/4/40], L vlPFC [BA 47], parahippocampal gyrus, as well as L amygdala, L midbrain, L orbital frontal cortex [BA 10], and L rostral anterior cingulate. **Conclusions:** A sexually dimorphic central network associated with SNS responses to a visceral stress can be identified in IBS patients. This network includes pain sensory areas as well as limbic and cortical regions involved in attention and affect. The sex-specific network may explain the previously noted differences in ANS responses between males and females. Sex related differences in ANS function may provide targets for future sex-specific therapies for this common chronic disorder.

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Role of spinal microglia activation in visceral hyperalgesia following chronic psychological stress in Wistar rats

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Background: We have previously demonstrated that repeated exposure to water avoidance stress (WA) leads to sustained exacerbation of the visceral nociceptive response to colorectal distension (CRD) for about 1 month. Microglia activation in the spinal cord has been implicated in sensitization of somatic nociception in several models of chronic pain. **Aims:** To determine if spinal microglia activation plays a role in visceral hyperalgesia observed after chronic WA stress. **Methods:** 1) To assess whether repeated WA or control sham WA (1hour daily, for 10 consecutive days) leads to activation of spinal microglia, lumbar spinal cord samples were collected 24 hours after the 10^{th} WA session (day 11) and were processed for immunohistochemistry analysis of microglia activation by labeling of OX42 marker. 2) We assessed the effect of a daily treatment with minocycline (inhibitor of microglia activation, 20 mg/kg, IP, daily, 1 hour before WA or sham WA) or vehicle (saline) on visceromotor response (VMR) to CRD at day 11 in WA and sham WA rats. **Results:** Quantitative (integrated density measurement) and qualitative (visual grading scores) analysis of OX42 immuno-staining showed activated microglia in WA rats compared to control. Integrated density in fixed area of dorsal spinal cord was significantly greater in WA group (121±7) compared with sham WA (69±4.2), arbitrary unit, $p<0.05$. Stressed rats treated with vehicle exhibited significant increase of VMR to CRD at day 11 compared with baseline ($P<0.05$), whereas minocycline treated rats failed to show visceral hyperalgesia indicating a preventive effect of minocycline on stress-induced visceral hypersensitivity to CRD. There was a significant difference in the effect of minocycline compared with vehicle at day 11 on the mean change from baseline for the pressure 40 mmHg (+4±14 versus +69±28 respectively) and 60 mmHg (+34±15 versus +119±54, respectively). Minocycline had no significant effect compared with vehicle, on the VMR to CRD in sham WA rats. **Conclusions:** The present data demonstrate the activation of spinal microglia after chronic WA stress and suggest a role of this activation in chronic stress induced visceral hyperalgesia. This is the first evidence for a role of spinal immune activation in the sensitization of visceral nociception by chronic stress.

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Gene expression analysis in rat amygdala in response to cisplatin (an emetic agent)

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Vomiting is a commonly encountered GI dysmotility problem involving antiperistalsis of gut contents and is often accompanied by nausea, a negative sensation involving processing of visceral information by higher cortical and subcortical CNS structures. Cancer chemotherapy agents like cisplatin are extremely potent for inducing nausea, vomiting and visceral malaise. Recent data from our laboratory showed increased Fos labeling in amygdala in response to cisplatin. These and other data suggest a role for the amygdala in integrating peripheral viscerosensory responses to generate aversive interoceptions like nausea. To explore the activity of amygdala in response to cisplatin, we used DNA microarrays to assess gene expression. Rats were injected (i.p.) with saline (n=3) or cisplatin (n=3, 10 mg/kg) and sacrificed at 24 h. Principal component analysis revealed no global effect of cisplatin on amygdalar gene expression. A two-class unpaired test revealed 2 genes whose expression increased and 298 genes where expression values were lowered after cisplatin treatment in comparison to saline-treated controls. Real-time PCR (n=6) validated the microarray findings and further *in silico* analysis showed diminished expression of key enzymes involved in biogenic amine synthesis (for example, tyrosine hydroxylase and dopamine decarboxylase) in the amygdala in response to cisplatin. These data hint at plasticity of serotonin/dopamine biosynthetic enzymes in amygdala in response to drugs like cisplatin which produce intense nausea and vomiting in emetic species.

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Studies of the CNS origin of NANC drive to the stomach

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Recent reviews of CNS control of gastric motility describe gastric relaxation as the product of simultaneous changes in brainstem vagal pathways. An excitatory cholinergic pathway is inhibited while a non-adrenergic, non-cholinergic (NANC) inhibitory pathway is activated. This inhibitory NANC pathway releases nitric oxide onto gastric smooth muscle. The purpose of these studies was to investigate the central origin of NANC drive to the stomach. To study this, an intragastric balloon was used to monitor intragastric pressure (IGP) in anesthetized rats. To eliminate excitatory cholinergic drive to the stomach, the animals were treated with atropine methyl bromide (0.1 mg/kg, i.v.). In five out of six animals, atropine methyl bromide consistently decreased IGP (−0.4±0.1 mmHg, $p<0.05$) and reduced the amplitude of phasic motility. In three animals, administration of atropine was followed by bilateral cervical vagotomy. Vagotomy in the presence of atropine produced an increase in IGP (+3.1±0.5 mmHg, $p<0.05$) that was significantly greater than vagotomy alone (+1.1±0.3 mmHg, $p<0.05$, n=12). These data suggested the presence of a tonic NANC drive originating from the dorsal motor nucleus of the vagus (DMV). To test this, rats were pretreated with atropine and L-gluta-

mate was microinjected into the DMV (rostral to calamus scriptorius). Microinjection of L-glutamate after atropine treatment produced no significant change in IGP (−0.1 ± 0.1 mmHg, n=4; p>0.05).

The failure to elicit gastric relaxation through activation of DMV neurons suggested that NANC drive may be maximal in the area rostral to calamus scriptorius. We hypothesized that if tone was maximal, inhibiting DMV neurons should increase IGP. To test this, muscimol (100pmol/30nl) was microinjected into the DMV of atropinized rats. The contralateral vagus was sectioned to remove efferent output from the contralateral DMV. In six out of seven animals, microinjection of muscimol caused an increase in IGP (+0.85±0.3 mmHg, p<0.05). To test if this increase in IGP was NANC-mediated, muscimol was microinjected in the presence of L-NAME (10mg/kg, i.v.), an inhibitor of nitric oxide synthase. Muscimol microinjected into the DMV of L-NAME-treated rats resulted in a decrease in IGP (−0.83±0.1 mmHg, n=6, p<0.05). These studies suggest the presence of a maximally active NANC drive that may originate in the intermediate DMV. Supported by RO1DK57105 (RAG) and NSO48746A (MTC).

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Electrophysiological study of spinal afferent nerves in the isolated rat pancreas

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The afferent innervation of the pancreas is poorly understood even though management of pancreatic pain is a major problem and sensory nerves contribute a neurogenic component to inflammation. **Aim:** To map and characterize spinal afferents in the rat pancreas. An isolated pancreas preparation, including the pancreatic duct (PD), common bile duct (CBD) and the splanchnic nerves was obtained from anaesthetized Sprague Dawley rats (n=14). The CBD was cannulated. The preparation was placed in a 2 chamber organ bath. One chamber accommodated the pancreatic tissue in physiological solution; the second chamber contained the nerve fibers in paraffin oil. Nerve recordings were obtained from the left splanchnic nerve. Stimuli consisted of probing (graded and non-graded), CBD/PD distension (44 mmHg), or electrical stimulation (ES; 20V, 0.5ms duration).

Discharge from 197 fibers was recorded, 57% displaying spontaneous activity. The mean discharge rate of spontaneously firing units was 2.52 ± 0.32 imp/sec. Sixty one mechanosensitive receptive fields were identified, mainly associated with arteries/blood vessels (33/61), particularly the gastric, splenic, and hepatic arteries, and the parenchyma (22/61). Receptive fields were also associated with pancreatic tissue near lymph nodes (4/61), CBD (1/61) and duodenal mesentery (1/61). 13% of the receptive fields received innervation from 2 or 3 separate nerve fibers in 7 preparations (70% of these located on blood vessels). All mechanosensitive responses were slowly adapting 33% displaying continuing discharge after termination of the stimulus. Graded responses from 27 units revealed that 60% of the units displayed a response threshold less than 10g. CBD/PD distension did not evoke any discharge from spontaneous or quiescent units. Non-mechanosensitive receptive fields, stimulated by ES only, were located in pancreatic tissue (7/17), arteries/blood vessels (9/17) or CBD/PD (1/17) and their function is yet to be established. Mechanosensitive nerves are associated with several structures in the pancreas, particularly the vasculature and parenchyma though, notably not with the major duct system. Continuing studies aim to establish the role of pancreatic afferents in physiological and pathophysiological signalling.

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Cross-talk between serotonin and substance P in gastrointestinal vagal afferent pathways in the rat

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Serotonin (5-HT) and substance P (SP) play major roles in the transmission of viscerosensory information in the gut-brain axis. Importantly, 5-HT and SP signaling contribute to the production of nausea and vomiting, and standard anti-emetic drugs target 5-HT and SP receptors. Here we investigate the potential cross-talk between 5-HT and SP signaling in vagal afferent transmission from the gut. In anethetized rats we recorded electrophysiological responses from the ventral trunk of the vagus, which receives a large afferent projection from the upper GI tract. 5-HT (20 ug/ml/kg) and SP (20 ug/ml/kg) were infused systemically into the jugular vein (j.v.). A multi-unit analysis (N=11, nerve recordings) revealed that 5-HT treatment increased vagal afferent activity by 250%. Similarly, SP treatment produced a 300% increase in multi-unit vagal afferent activity. A 5-HT_3 receptor antagonist (Y-25130, 0.8 mg, j.v.) substantially reduced the immediate effects of 5-HT-induced multi-unit activation of vagal afferent fibers (~70% reduction in 30 sec after start of 5-HT infusion) but had no effect on SP-induced activation. To compare the activation of vagal fibers by 5-HT and SP single units were selected based on cluster analysis of principal component scores and template matching. This analysis (N = 30) revealed a category of vagal afferent fibers that was sensitive to both 5-HT and SP (43% of total). Other categories included single afferent fibers sensitive to only 5-HT (17%) or SP (23%). 17% of single units were not affected by either treatment. In summary, these experiments reveal GI sensory fibers that show heterogeneous esponses to 5-HT and SP. Further delineation of the pharmacology of these nerve fibers might suggest novel ways to target nausea and vomiting.

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A novel model to study gastric accommodation and motility in conscious rats

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Introduction: Intragastric pressure (IGP) measurement is normally used to study gastric motor activity. However, during gastric distension IGP can also be used to examine gastric accommodation. We set out to optimise the technique of IGP measurement during gastric filling in order to quantify both gastric accommodation and motility in conscious rats. To validate the model effects of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) and the muscarinic receptor antagonist atropine were examined. **Methods:** Six female Sprague Dawley rats were used in control experiments and after subcutaneous injection of 30 mg/kg L-NAME as well as 1 mg/kg atropine. After an overnight fast, a test-meal infusion system and a catheter to measure IGP were connected to a previously implanted gastric fistula. Following a 15 min stabilisation period, the non-nutritious viscous test-meal (containing 0.5gr barium sulphate/ml in a 3% hydroxypropyl methylcellulose solution) was infused until 20 mmHg IGP or a volume of 15 ml was infused. Peristaltic waves were associated with IGP waves (visual observation from X-ray images recorded simultaneously). A motility index (MI) was calculated for each IGP wave associated with peristaltic motility as wave amplitude divided by wavelength. The underlying IGP trend was used to study gastric accommodation. Results were expressed as mean±SEM; MI's were compared with repeated measurements ANOVA (P<0.05 considered significant). **Results:** In control experiments IGP increased linearly to 11.2±0.6 mmHg at 4.0 ml infused and remained stable at this level during further infusion. Both after L-NAME and atropine treatment IGP increased during the whole test-meal infusion to a maximum of 20.7±0.6 mmHg at 11.5 ml and 14.1±0.7 mmHg at 15 ml infused respectively. Atropine significantly decreased MI's compared

to control experiments. L-NAME significantly increased MI's compared to control experiments when MI's were grouped per ml infused test-meal. However, no differences between L-NAME treatment and control was seen when MI's instead were grouped per mmHg IGP increase.

Discussion & Conclusion: For the same intragastric content IGP was higher after L-NAME and atropine treatment compared to control experiments indicating that both drugs impair gastric accommodation. Atropine decreased motility-induced IGP waves, indicating that it reduces peristaltic motility. The effect of L-NAME on gastric motility was however ambiguous showing either significant or no effect on IGP waves associated with motility depending on the analysis. IGP measurement during gastric filling has proved to be useful to study gastric accommodation and peristaltic motility in conscious rats.

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Neurokinin NK2 receptors are involved in mediating stress-induced fecal pellet output and ACTH secretion in gerbils

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Introduction: The neurokinin (NK) receptors are involved in the regulation of intestinal motility and play a role in mediating behavioural and autonomic responses to stressors in experimental animals. Previous studies have demonstrated that NK1 receptors in particular mediate stress-induced defecation in rats and gerbils. The aim of this study was to investigate the effects of the selective NK2 receptor antagonist SR48968 on stress-induced fecal pellet output and ACTH secretion in gerbils. **Methods:** Male Mongolian gerbils 10–12 weeks of age were used. Gerbils were habituated to grid-wire-bottomed cages five times before stress experiments. Acute stress was induced by placing gerbils in narrow plastic cylinders (i.e. Bollmann cages) for 60 min restricting their capacity to move. Gerbils placed in wire-bottomed cages served as non-stressed controls. The amount of fecal pellets defecated during the experiment were counted. Some animals were sacrificed immediately after the experiments and plasma ACTH levels were measured using radioimmunoassay. Compound or corresponding vehicle were administered intraperitoneally (ip) 30 min prior to the stress paradigm. **Results:** Acute stress increased fecal pellet output more than 2-fold compared to non-stressed controls (stress: 12.5±0.9, control: 5.6±0.7, $p<0.001$). ACTH levels also increased 2-fold in response to stress (stress: 3906±334 pg/ml, control: 2171±477 pg/ml, $p<0.05$). The selective NK2 receptor antagonist SR48968 decreased stress-evoked pellet output in a dose-dependent manner with doses of 1 and 3 μmol/kg reducing pellet output to control levels (5.2±1.4 and 6.6±1.5 respectively). SR48968 (3 μmol/kg) also reduced stress-evoked ACTH levels to control levels (2320±520 pg/ml). **Conclusion:** NK2 receptors participate in mediating the effects of stress on colon motility and ACTH secretion in gerbils.

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Rectal compliance and neurohormonal-immune signaling in diarrhea-predominant irritable bowel syndrome

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Irritable bowel syndrome (IBS) is regarded as a disorder of brain-gut axis regulation. Abnormal rectal compliance seems a consistent feature of diarrhea-predominant (d-) IBS. The serotonergic system, HPA-axis, and immune system are all integrated within the brain-gut concept.

The aim of this study was to investigate differences in rectal compliance and its relationship to biochemical parameters of serotonergic metabolism, HPA axis and immune activation, respectively, in patients with d-IBS and healthy controls. **Methods:** (i) Platelet poor plasma 5-hydroxytryptamine (ppp 5-HT), its metabolite 5-hydroxyindole acetic acid (5-HIAA), 5-HT turnover (5-HIAA:5-HT), platelet 5-HT, (ii) salivary cortisol and, (iii) serum levels of the proinflammatory cytokine TNF-α were used as parameters of serotonergic metabolism, HPA axis and immune activation, respectively, in 22 d-IBS patients and 22 matched (age, sex, BMI) healthy controls. Rectal compliance (pressure-volume relationship) was assessed using a barostat procedure. Subsequently, group differences were analyzed by independent samples t tests and associations between the biochemical parameters and rectal compliance were analyzed using linear regression in the entire study population. **Results:** The d-IBS patients showed significantly increased ppp 5-HT levels (d-IBS 11.8 ± 1.3 nmol/L; controls 8.2 ± 0.8 nmol/L, $p = 0.03$), decreased 5-HIAA levels (d-IBS 29.1 ± 1.0 nmol/L; controls 32.9 ± 1.5 nmol/L , $p = 0.03$), decreased 5-HT turnover (d-IBS 3.45 ± 0.5; controls 4.88 ± 0.5, $p = 0.04$) and reduced rectal compliance (d-IBS 4.6 ± 0.5 ml/mmHg; controls 7.0 ± 0.5 ml/mmHg, $p = 0.002$), compared to controls. The levels of ppp 5-HT ($F = 5.2$, $p = 0.03$, $R^2 = 0.11$), cortisol ($F = 5.9$, $p = 0.02$, $R^2 = 0.12$) and TNF-α ($F = 5.5$, $p = 0.02$, $R^2 = 0.12$) correlated significantly with reduced rectal compliance. Ppp 5-HT was significantly positively associated with cortisol levels ($F = 9.4$, $p = 0.004$, $R^2 = 0.18$). **Conclusions:** We demonstrated that reduced rectal compliance in d-IBS is significantly related to alterations in neurohormonal and immune signaling. This study may facilitate the search for biomarkers of integrated brain-gut function in IBS.

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Differential activation of NTS subnuclei by stimulation of different esophageal mechanoreceptors

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Aim: To determine whether activation of different esophageal reflex pathways activates different sets of NTS subnuclei. **Methods:** Cats (N=27) anesthetized with alpha-chloralose (50mg/kg, IP) were instrumented to stimulate the cervical esophagus in three ways: 1) Slow muscle distension; sinusoidal 3/min polyvinyl balloon (3–4cm long) inflated to 40–50mmHg, Rapid mucosal touch; rapid (0.2s) air pulse of 8mmHg directed tangentially 3/min, and 3) Slow mucosal/submucosal tension; rotation 8/min of latex rubber balloon (3–4cm long) filled to relaxed diameter (8–10mm) of esophagus. Brains of stimulated and control cats were removed after 3 hrs for *c-fos* immunohistochemistry, and 6 of these were also instrumented to record from pharyngeal and laryngeal muscles and esophagus to characterize the responses. Anesthesia was used to block secondary peristalsis that itself could activate esophageal reflexes. **Results:** We found that slow muscle distension activated the UES and relaxed the LES; rapid mucosal touch activated laryngeal and hyoid muscles similar to a belch response; and slow mucosal/submucosal tension caused pharyngeal swallows. None of the stimuli activated esophageal peristalsis The three different esophageal stimuli also activated three different sets of NTS subnuclei (see Table below). Values are numbers of *c-fos* positive neurons. NTS, nucleus tractus solitarius; *, $P<0.05$, t-test vs. control. **Conclusions:** We conclude that 1) the esophagus is controlled by at least three different sets of NTS premotor nuclei; 2) prior tract tracing studies delineated NTS pathways of esophageal muscle afferents only and not mucosal or submucosal afferents; 3) NTSce probably mediates responses from the slowly adapting muscle tension receptors; 3) the NTSvl, NTSd, and AP probably mediate responses from rapidly adapting mucosal touch receptors; 4) the NTSis, NTSim and NTSd probably mediate responses from slowly adapting mucosal/submucosal tension receptors; 5) the AP may be involved in the control of belching as well as vomiting; and 6) the swallow inducing mucosal tension receptors of the pharynx may extend into the esophagus as activation of either activates the same responses and same NTS premotor nuclei.

NTS/*Stim*	N	Central	Interstitial	Intermediate	Dorsal	Medial	Ventrolat	AP
Control	5	17 ± 3	9 ± 2	11 ± 2	7 ± 2	8 ± 3	5 ± 2	7 ± 2
Slow Dist	5	**62 ± 10***	9 ± 2	10 ± 2	10 ± 2	5 ± 2	8 ± 2	5 ± 2
Rapid Dist	5	21 ± 6	8 ± 2	7 ± 2	**22 ± 4***	6 ± 2	**24 ± 4***	**84 ± 20***
Rotation	6	22 ± 3	**28 ± 5***	**24 ± 4***	**32 ± 7***	6 ± 2	11 ± 4	13 ± 3

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Activation of peripheral CRF_1 receptors with cortagine reproduces stress-related stimulation of colonic motor function and visceral hyperalgesia in conscious rats

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Background: CRF binds to CRF receptor 1 (CRF_1) and with a lesser affinity to 2 (CRF_2). Selective activation of peripheral CRF_2 inhibits gastric emptying and dampens visceral motor response (VMR) to repeated colorectal distention (CRD) (Gut 2006;55:172-81). CRF stimulates colonic motor function though CRF_1 and has no effect on VMR to CRD (Br.J.Pharmacol. 2004;141:1321-30, Peptides 2005;26:1188-95). Whereas highly selective agonists for CRF_2 exist, no agonist with a similar selectivity for CRF_1 was available until the recent development of the new CRF_1 agonist cortagine (PNAS 2004;101:9468-73). **Aim:** To assess whether selective activation of peripheral CRF_1 receptor with intraperitoneal (IP) cortagine reproduces stress-related colonic motor and visceral hyperalgesic responses. **Methods:** Conscious male SD rats (250–300g) were used except otherwise stated. The fecal pellet output (FPO) and diarrhea incidence were monitored 1 h after cortagine (3 and 10 μg/kg IP). The specific CRF_1 antagonist CP154,526 (20 mg/kg, SC) was injected 30 min before cortagine (10 μg/kg, IP). Gastric emptying of solid food was measured 4 h after cortagine (10 μg/kg IP). cFos IHC on colonic whole-mount preparations myenteric neurons was assessed 60 min after cortagine (10 μg/kg IP). In female SD rats equipped with electrodes for electromyographic (EMG) recording, the VMR to phasic CRD (10, 20, 40, 60 mmHg; 20s duration, 4 min inter stimulus interval) was monitored. One hour after a baseline CRD, rats were injected with cortagine (10 μg/kg IP) or vehicle and after 15 min a second CRD was performed. **Results:** Cortagine at 10 μg/kg, but not 3 μg/kg, IP, significantly increased the FPO and induced diarrhea in 43% of rats within 30–45 min post-injection. Cortagine responses were prevented by CP-154,526. By contrast, cortagine had no effect on gastric emptying. Cortagine induced cFos expression in the myenteric neurons of proximal and distal colon (7.8±0.6 and 9.2±0.6 cFos-IR cells/ganglion, vs vehicle 0±0 and 0±0, respectively). Cortagine also exacerbated the VMR to CRD in all volumes of CRD compared to baseline reaching significance at 60 mmHg in female rats. **Conclusions:** Selective activation of peripheral CRF_1 signaling pathways by cortagine reproduces features of irritable bowel syndrome-diarrhea predominant by inducing a short onset of defecation, diarrhea, visceral hyperalgesia to CRD and colonic myenteric activation providing new models to study the pathophysiology of IBS. Supported by R01 DK33061.

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Activation of vagal A-type afferents inhibit visceral pain perception

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Peripheral serotonin (5-HT) has been implicated to transmit visceral nociception to the CNS. In this study, we first investigated whether luminal 5-HT activates both C- and A- type vagal afferent fibers and examined whether 5-HT sensitive vagal afferents contain VR1 and P2X receptors. Single neuronal discharges of nodose ganglia neurons innervating the duodenum were recorded *in vivo* in rats, followed by juxtacellular neurobiotin labeling to characterize VR1 and P2X receptor expression. Vagal A- and C- type neurons were identified based on conduction velocities. Luminal perfusion of 5-HT (10^{-5}M) elicited an increase in vagal C- and A- type neuronal discharges in 17/30 and 11/36 neurons recorded respectively. Double labeling immunocytochemistry showed that all of the 9 C-type neurons labeled by neurobiotin contained VR1 receptors. In contrast, while 7 labeled A-type neurons activated by 5-HT contained P2X receptors, none of them expressed VR1 receptors. Previously, we have shown that lower intensity electrical vagal stimulation which activates capsaicin-insensitive A-fibers regulates CNS cortical activities stimulated by noxious colorectal sensation. Here, we examined the hypothesis that subdiaphragmatic vagal A-type nerves play a role in the modulation of visceral pain. Visceromotor responses electromyographic (VMR) to tonic colorectal distension (CRD) were examined in conscious rats. The response was considered stable if there was less than 10% variability between colonic distensions (80 mmHg) of two consecutive trials. CRD (20, 40 and 80 mmHg) produced reliable and reproducible contractions of the lateral abdominal musculature (from basal 0 to 3 ± 0.5, 22 ± 3, 45 ± 6 per 5 seconds, respectively). High intensity subdiaphragmatic vagal afferent stimulation (400 μA, 20 Hz, 0.5 ms; 1 minute prior to CRD) which activated C-type fibers had no effect on CRD induced VMR. In contrast, low intensity electrical vagal stimulation (40 μA, 20 Hz, 0.5 ms, 1 minute prior to CRD) which activated vagal A-type fibers reduced CRD-induced abdominal muscle contractions to 0, 15 ± 3 and 21 ± 4 per 5 seconds, respectively. This response was not affected by perivagal capsaicin-treatment. We conclude that intestinal 5-HT differentially activates subpopulations of both A- and C- type vagal primary afferent neurons. Most of 5-HT activated vagal A-type neurons also contain P2X receptors sensitive to ATP which may be released during intestinal muscle contraction or inflammation. This is the first demonstration that electrical stimulation of subdiaphragmatic vagal A-type nerves suppresses experimentally induced visceral pain.

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Visceral hyperalgesia following intralcolonic TNBS in rats can be prevented by the TRPV1 receptor antagonist JYL-1421

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Background: Visceral hyperalgesia may result from colonic inflammation. The transient receptor potential vanilloid 1 (TRPV1) receptor may play an important role in visceral nociception and neurogenic inflammation. We aimed to examine the effects of the TRPV1 receptor antagonist JYL-1421 on visceral hyperalgesia and inflammation in a rat model of colitis. **Methods:** Acute inflammation was induced in male Sprague-Dawley rats by single administration of TNBS (trinitro benzenesulfonic acid) in the distal colon. In all rats, a baseline visceromotor response (VMR) to graded colorectal distension (CRD, 10–80mmHg, 30s, 180s interstimulus intervals) was recorded prior to TNBS. Rats were randomized to receive pre-emptive JYL-1421 (10μmol/kg) or saline. JYL-1421 was given intravenously 15 minutes prior to administration of TNBS followed by daily doses for 7days. Control rats received i.v. saline on the same schedule. Seven days post-TNBS, the VMR was repeated and compared to baseline and controls. In a different group, daily JYL-1421 (10μmol/kg, i.v.) or saline was given one week following intracolonic TNBS. The VMR was recorded prior to JYL (1 week post-TNBS) and after 7 days of treatment. To assess the severity of inflammation, sections of the distal colon were examined with H&E staining under light microscopy and myeloperoxidase (MPO) activity. **Results:** In the control group, intracolonic TNBS produced a significant increase in the VMR to CRD at pressures > 10mmHg (n=18, p=0.01). However, rats that received pre-emptive JYL-1421 failed to demonstrate any alteration in the VMR when compared to baseline (n=16, p>0.05). The VMR of control rats was significantly higher than that of the JYL-1421 treated group at distension pressures > 10mmHg, (p<0.02). In control rats (n=4), TNBS resulted in an increase in submucosal neutrophilic infiltrates that was markedly lower in the treated group (n=4). The mean MPO activity was significantly lower in the JYL -1421 treated group (7.98±3.0, n=6) when compared to controls (33.47± 8.6, n=5) (p=0.01). In the post-emptive groups, the VMR was significantly higher than baseline seven days following TNBS. At the end of JYL-1421 treatment, there was a significant decrease in the VMR (CRD=30mmHg) when compared to controls. No significant difference was observed between groups in the MPO activity (JYL-1421 0.94±0.57 vs saline 1.80±.68, p>0.05). **Conclusion:** Systemic administration of the TRPV1 antagonist JYL-1421 attenuates the colonic inflammation and visceral hyperalgesia resulting from TNBS-induced colonic inflammation.

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Does ghrelin increase during nausea?

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The appetite stimulating hormone ghrelin is mainly secreted from the stomach was found to reduce emesis in a chemotherapy induced nausea model in ferrets when given intra-peritoneally. The mechanism of its anti-emetic activity is not yet clear. Therefore, we investigated ghrelin levels during motion sickness induced nausea. **Methods:** Eight healthy male subjects (24.8±1.4 years) being susceptible to vection were recruited to our test procedure. To induce nausea a slow rotation procedure (120° per sec) around the vertical axis for 1 min with head movements was used. After a break (1 min) rotation was repeated until nausea forced the subject to stop. Standardized nausea ratings (using a sum score) and blood sampling was performed before and immediately after the rotation procedure. Plasma levels of ghrelin, ACTH, antidiuretic hormone (ADH), and pancreatic polypeptide (PP) were determined by radioimmunoassays. **Results:** During the rotation period each subjects developed nausea. Rotation time was between 60 an 300 seconds (145.9±29.6 seconds). ACTH levels (from 53.9±13.1 to 124.6±24.0 pg/mL) and ADH (from 0.4±0.04 to 20.2±5.3 pg/mL) were both significantly increased after the rotation period (P=0.012). PP was also significantly increased during nausea development (from 12.6±2.7 to 18.0±2.6 pmol/L; P=0.05). In contrast, ghrelin levels did not change significantly (before rotation: 387.1±35.2 pg/mL; after rotation: 382.3±36.7 pg/mL). **Conclusions:** While ACTH, ADH, and PP were significantly increased, no endogenous ghrelin release was found during motion sickness induced nausea. To understand the ability of ghrelin to reduce chemotherapy induced nausea merits further investigations.

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Activation of the brainstem nucleus tractus solitarius inhibits the activity of the internal and the external esophageal sphincters in the ferret

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It is well documented that the nucleus tractus solitarius (NTS) of the brainstem plays a seminal role in regulating the behavior of the dorsal motor nucleus of the vagus (DMV), which serves as a primary source of preganglionic innervation to the lower esophageal sphincter (LES) and the gastrointestinal tract (GI). Recently, we reported that stimulation of the DMV can affect both the internal and external esophageal sphincters in a site specific manner, and that inhibition of the internal sphincter is mediated by both nitric oxide and vasoactive intestinal polypeptide (VIP) (Niedringhaus, et al., 2005; Sahibzada, et al., 2006). To elaborate further on this brain-gut interaction, we examined the role of the NTS in regulating the activity of both these sphincters at the esophagogastric junction. Experiments were performed in isoflurane anesthetized male ferrets whose LES pressure and gastric motility were monitored by a dentsleeve catheter and a strain gauge transducer (sutured onto the fundus), respectively. A bipolar platinum electrode was employed to record the electromyographic (EMG) activity of the crural diaphragm (CD; external sphincter). To activate NTS neurons, L-glutamate (500pmol/30nL) was microinjected unilaterally into the NTS +0.5mm rostral to the calamus scriptorius (CS), a distance equivalent (vis-à-vis the rostrocaudal axis) to our previous studies. L-glutamate microinjected into the NTS elicited a relaxation (in reference to baseline) of both the LES pressure (−12.7mmHg) and the gastric tone (−0.34g). Additionally, the frequency of EMG activity of the CD was decreased (−5.5 bursts/min). Vagotomy abolished the LES response to L-glutamate. A combination of L-NAME (10mg/kg; IV) and VIP_{6-28} receptor antagonist (0.350mg/kg; IV) abolished L-glutamate induced decreases in LES pressure, but had no effect on its decreases in EMG activity and stomach contractility. Pretreatment with atropine methyl bromide (0.1mg/kg; IV) was successful only in abolishing the L-glutamate decreases in fundus contractility, whereas the other responses were unaffected. Our preliminary data indicate that similar to DMV stimulation: (1) NTS activation can inhibit the functional activity of both sphincters in addition to decreasing gastric tone, and (2) inhibition of the internal sphincter due to excitation of the NTS is mediated by a non-cholinergic-non-adrenergic pathway. Supported by NIH Grant DK56920.

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Previous inflammation increases the susceptibility of the colon to the effects of subsequent psychological stress

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Stress has been associated with the exacerbation of symptoms in functional gastrointestinal disorders such as Irritable Bowel Syndrome (IBS). In addition some patients that suffer from gastroenteritis develop IBS-like symptoms. **Aim:** To measure the effects of previous intestinal inflammation on stress-induced changes in epithelial transport, membrane integrity and colonic damage. **Methods:** Four groups of female rats were used: normal (untreated); control-WAS (water avoidance stress), where animals received stress alone; control-colitis, where animals received trinitrobenzene sulfonic acid (TNBS 30mg in 50% ETOH ic) to induce an acute colitis which was then allowed to heal for 2 weeks; and colitis-WAS, where the animals received TNBS as above, then were subjected to WAS 2 weeks later. WAS was induced by placing the animals on a raised platform in a plastic container filled with water. The stress protocol consisted of 3 hrs of stress per session, during 3 consecutive days. To measure anxiety-like behaviour, animals were placed in an open field arena prior to start of the stress protocol, and 30 min after the last session of stress. The animals were sacrificed on the day after the last stress session. All animals were evaluated for weight change, presence of diarrhea, propulsive and myeloperoxidase (MPO) activity, macroscopic and microscopic damage. Tissues from the distal colon were stripped of muscularis propria and mounted in Ussing chambers for the continuous measurement of short circuit current in response to acetylcholine. **Results:** Stress increased weight loss in animals that previously had colitis compared to controls ($p<0.05$). These animals also had greater colonic propulsive activity. Stress increased MPO levels, and the macroscopic and microscopic damage in the colonic tissue compared to controls. WAS also significantly decreased the ability of these colonic tissues to respond to acetylcholine compared to controls ($p<0.05$). WAS in animals which did not have previous inflammation did not cause changes in macroscopic or microscopic damage, MPO levels, or response to acetylcholine. These animals did however show increased colonic propulsive activity ($p<0.05$) and weight loss ($p<0.05$) compared to normals. There was a decrease in locomotion and normal behaviour, such as grooming, in colitis-WAS rats compared with control-WAS. **Conclusion:** Prior inflammation increases the susceptibility of the colon to respond to stress, affects epithelial barrier function and increases anxiety-like behaviour. *Supported in part by 2G-12RR03050.*

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Acute stress induced hypersensitivity to rectal distension depends upon increase serine protease activity in rats : involvement of CRF

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We have recently shown that the increase of colonic permeability induced by partial restraint stress (PRS) is responsible for stress-

induced rectal hypersensitivity. Moreover proteinase-activated receptor agonist (SLIGRL) increases gut paracellular permeability in mice. The aims of this study were to determine whether 1) PRS modify, via a CRF pathway, fecal and colonic serine protease activity in rats; 2) rectal hypersensitivity induced by a PRS is linked to serine protease activity changes. **Methods:** Groups of female Wistar rats were used. Protease activity (PA) and Rat Mast Cell Protease II (RMCPII) levels were determined in both fecal pellets and colonic content in rats submitted to 1) IP CRF (50 μg/kg) administration, 2) a PRS during 2 h, 3) a PRS plus α-helical CRF 9-41 (250 μg/kg IP) or SSR-125543 (10 mg/kg IP) or vehicles pre-treatment. The abdominal cramps reflecting visceral pain were assessed in rats previously fitted with an intracolonic catheter and equipped with electrodes implanted in the abdominal striated muscle. Four hours before and 20 min after PRS, a RD was performed using a Fogarty probe inflated from 0 to 1.2 ml in rats intracolonically infused with Nafamostat (a serine protease inhibitor, 0.5 mg/rat) or vehicle (NaCl 0.9%) 1 h before and during the PRS session. **Results:** Compared to control, CRF as well as PRS significantly increased PA in both fecal (0.92 ± 0.25; 1.10 ± 0.18 *vs* 0.40 ± 0.12 UA trypsin/mg protein) and colonic content (0.80 ± 0.13; 0.89 ± 0.20 *vs* 0.43 ± 0.09 UA trypsin/mg protein) in rats. A previous treatment by α-helical CRF 9-41 or SSR-125543 significantly suppressed the PRS-induced colonic/fecal contents PA increase. In contrast, no changes in RMCPII levels determined in fecal pellets or colonic contents in the different groups of animals were observed. PRS significantly increased ($p<0.05$) the number of abdominal cramps for all RD volumes applied reflecting colonic hypersensitivity. Intracolonic infusion of Nafamostat supressed ($p<0.05$) the increase in number of abdominal cramps induced by PRS, for all RD volumes. **Conclusion:** The increase of colonic serine protease activity induced by stress is mediated by CRF release and participated through altered permeability to stress-induced rectal hypersensivity in rats.

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Altered gastric emptying and brain catecholaminergic pathway in fasted CRF-overexpressing mice

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Background: Corticotropin-releasing factor (CRF)-overexpressing (OE) mice display endocrine, behavior and visceral features of chronic stress. **Aim:** To investigate gastric emptying (GE) and brain Fos response to abdominal surgery in fasted CRF-OE mice. **Methods:** The GE of a non-nutrient viscous liquid was determined in CRF-OE and wild type (WT) mice fasted for night, or operated with laparotomy and cecum palpation or sham surgery. Brains from the same mice were processed for Fos immunoreactivity (ir) and double staining with tyrosine hydroxylase (TH). **Results:** CRF-OE mice fasted overnight had higher basal GE than WT mice (83.2±0.8% v 48.9±3.9%; $P<0.05$) and developed delayed GE after abdominal surgery that had similar magnitude of decrease from respective basal values (Δ changes: 33.4% in CRF-OE v 30.7% in WT). In sham-operated mice, Fos-ir in the locus coeruleus (LC) and ventrolateral medulla (VLM) of the brain was significantly higher in CRF-OE than WT mice (Table) and those neurons were double-stained with TH-ir (about 95% in the LC and 55% in the VLM). Abdominal surgery further increased Fos-ir in the LC of CRF-OE mice by 2.9 and 2.3 folds compared with WT and CRF-OE sham-operated mice. Fos-ir cells in the nucleus tractus solitarius (NTS) was increased significantly only in operated CRF-OE mice. There was no difference of Fos-ir between WT and CRF-OE in the paraventricular nucleus of the hypothalamus (PVN) and VLM induced by abdominal surgery. Changes of Fos-ir in the supraoptic nucleus (SON) and dorsal motor nucleus of the vagus were not significantly different. **Conclusions:** Fasted CRF-OE mice display increased basal gastric emptying and activation of hindbrain catecholaminergic neurons, and respond to abdominal surgery by a stronger activation of LC and NTS than wild type mice. Supported by NIH DK 33061, NIH DK 64539 and VA Merit review/VA.

Table 1 Brain Fos-ir (mean±SEM cells/section)

Genotype	Treatment	PVN	SON	LC	VLM	NTS	DMN
WT	Sham	24.4±9.5	31.5±10.9	5.4±1.7	5.1±1.5	35.7±5.9	0.6±0.3
CRF-OE		19.3±2.7	42.2±8.4	26.0±8.1⋆	13.5±1.5⋆	45.5±6.1	8.7±4.0
WT	Abdominal Surgery	111.6±25.9⋆	69.7±16.6	21.7±6.8	14.6±1.4⋆	56.7±12.3	1.1±0.3
CRF-OE		111.6±15.9⋆	78.0±10.3	84.5±7.7⋆#	16.6±2.1⋆	87.5±15.9⋆	8.1±3.8

⋆: $P<0.05$ vs WT sham; #: $P<0.05$ vs WT abdominal surgery.

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Genetic differences determine inflammatory response and development of visceral hyperalgesia in a rat model

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Background: Transient inflammation is a risk factor for the manifestation of Irritable Bowel Syndrome (IBS) and can cause long- lasting visceral hyperalgesia. Although GI infections are common, only a group of patients develop post-infectious IBS. We therefore hypothesized that genetically determined differences influence inflammatory response and visceral sensory function. **Aim:** To characterize the inflammatory response and acute and long-term effects of a transient colitis on visceral sensory function in two rat strains. **Methods:** Colitis was induced by single colorectal instillation of Trinitrobenzenesulfonic acid (TNBS; 5 mg/kg) plus ethanol or saline (control) in male Fisher344 (F) and Lewis (L) rats. At 2 hr and 5, 14 and 28 days after induction of colitis colonic tissue samples were histologically assessed and blood samples assayed for IL–2 plasma levels via ELISA. A subset of rats was injected iv. with ^{99m}Tc-labelled leukocytes (LWC) for qualitative (images) and quantitative (bio-distribution) scintigraphic evaluation of colitis at the various study days. Visceromotor response (VMR) to colorectal distensions was assessed before and 5, 14 and 28 days after induction of colitis. **Results:** TNBS caused an acute transient inflammation in both strains with a peak at day 5 and histological healing at day 14. Compared to L rats inflammatory response was significantly ($p<0.05$) attenuated in F rats based on histology and LWC assay. IL-2 was increased in both strains prior to occurrence of mucosal lesions and remained elevated at day 14 in L but not in F rats. VMR to CRD was significantly enhanced ($p<0.01$) on day 5 in TNBS treated rats (L+F) compared to saline controls and returned to baseline level at day 14. At day 28 VMR was significantly increased in L rats but not in F rats. **Conclusion:** Compared to F rats, L rats develop a long lasting visceral hyperalgesia and more severe inflammation following TNBS instillation. Thus the presumably genetically determined difference in the susceptibility for a post-inflammatory visceral hyperalgesia appears to be associated with the intensity of the mucosal inflammation and the systemic immune response.

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Effects of 5-HT$_3$ antagonists on symptom relief and constipation in irritable bowel syndrome: a systematic review and meta-analysis of large, multicenter, randomized trials

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Background: Large, randomized trials have demonstrated the efficacy of 5-HT$_3$ antagonists in patients with non-constipated or diarrhea-predominant irritable bowel syndrome (NC- or D-IBS respectively). However, the benefit-risk ratio remains unclear. We performed a systematic review and meta-analyses to estimate the overall efficacy and constipation rate of the drug class of 5-HT$_3$ antagonists in NC- or

D-IBS patients. **Methods:** We searched for published reports of randomized, placebo-controlled trials of 5-HT_3 antagonists using a comprehensive computer-based search of MEDLINE, EMBASE, and Web of Science. Using a random effects model, we pooled the results across eligible trials and estimated the relative risks (RR) and number-needed-to-treat (NNT) or –harm (NNH) respectively for relief of abdominal pain and discomfort, for global improvement of IBS symptoms, and for the development of constipation. **Results:** Two independent investigators (VA, JK) identified 14 eligible trials that fulfilled pre-set criteria for high quality and relevant clinical endpoints. One trial assessed only safety. 3024 patients were randomized to alosetron (A) 1 mg bid; 1116 to cilansetron (C) 2mg tid; 3043 to placebo; and 304 to mebeverine 125 mg tid as comparator. Table 1 shows meta-analytic estimates for the different endpoints. Similar treatment effects were documented in all studies and efficacy was significant for both alosetron and cilansetron and for both men and women. There were 9 cases of at least possible ischemic colitis in the treatment group (0.2%) and 0 in the control group. **Conclusion:** As a class, 5-HT_3 antagonists significantly improve abdominal pain and discomfort and global IBS symptoms in men and women with NC- or D-IBS. Treatment with 5-HT_3 antagonists is associated with an increased risk of constipation, but the risk is lower in patients who report predominantly diarrhea.

Table 1: (* the 3 C studies reported both efficacy endpoints)

Endpoints	No. of studies	RR [95% CI]	NNT/H [95% CI]
Relief of abdominal pain and discomfort	10 (7 A, 3 C*)	1.31 [1.24, 1.39]	8 [6, 10]
Global improvement of IBS symptoms	6 (3 A, 3 C*)	1.58 [1.41, 1.76]	5 [4, 7]
Constipation overall	14 (11 A, 3 C)	4.28 [3.28, 5.60]	5 [4, 7]
Constipation in NC- and D-IBS	6 (6 A, 0 C)	5.58 [4.27, 7.30]	6 [4, 8]
Constipation in D-IBS only	8 (5 A, 3 C)	3.60 [2.56, 5.05]	8 [5, 13]

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Values are presented as mean ± SD

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Alterations in expression of p11 (S100A10) in mucosal biopsies of patients with irritable bowel syndrome

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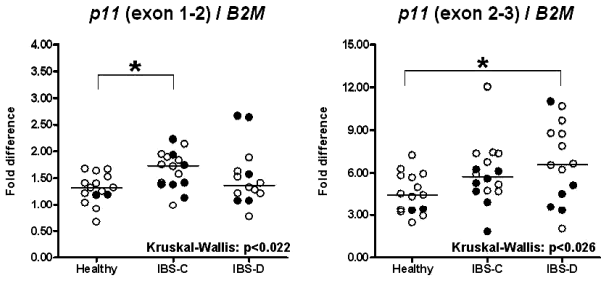
Background/Aim: Abnormalities in serotonin (5-HT) metabolism are reported in irritable bowel syndrome (IBS). The protein p11 (*S100A10*) influences the response to agents that modulate 5-HT and is decreased in depression, a frequent co-morbid condition in IBS. We hypothesized that expression of p11 in colonic mucosa is abnormal in patients with IBS as compared to healthy controls. **Methods:** p11 mRNA expression was measured by RTQ-PCR in sigmoid and rectal mucosal biopsies from well characterized IBS patients whose symptoms were evaluated within 1 week of biopsies. RTQ-PCR was performed for p11 (exons 1 and 2), p11 (exons 2 and 3) and a control gene with similar level of tissue expression, beta-2 microglobulin (B2M). **Results:** p11 expression in sigmoid mucosal biopsies was increased in IBS (fig); when IBS-D (n=14) and IBS-C (n=16) patients were pooled into one group of IBS patients and compared to healthy controls (n=15), p11 mRNA was significantly increased relative to controls for both RTQ-PCR assays (* in figures, p<0.009 and p<0.028 respectively for p11 exon 2-3 and p11 exon 1-2). Rectal biopsies showed no differences in p11 mRNA expression. Assay for p11 exons 1 and 2 or for p11 exons 2 and 3 revealed significantly increased expression in IBS-C and IBS-D patients respectively compared to controls. p11 mRNA was not affected by antidepressant treatment (filled circles in figures) in any of the analyzed subgroups. **Conclusion:** p11 expression in sigmoid mucosal biopsies was increased in IBS. Since over-expression of p11 can increase serotonergic receptor functions (e.g. 5-HT1B receptors), further study of the interaction between p11 expression in health and disease is needed. Grant support: J&J Pharmaceutical R&D and NIH RO1 DK 54681(MC).



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Altered gastrointestinal motility and colonic damage contribute to disease symptoms in an animal model of intestinal endometriosis

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Intestinal endometriosis commonly presents with gastrointestinal symptoms that mimic Inflammatory Bowel Disease (IBD) and Irritable Bowel Syndrome. However, little is known about the pathophysiological mechanisms at play. While IBD is associated with changes in intestinal motor function that correlate with severity, no such information is yet available for endometriosis. **Aim:** To correlate motility changes with colonic damage and inflammation in an animal model of intestinal endometriosis. **Methods:** Intestinal endometriosis was surgically induced by suturing four uterine horn implants beside the mesentery of the small intestine in female Sprague-Dawley rats for 60 days. Sham-control animals received sutures only. A group of normal weight-matched animals was also included. Cytologic smears were carried out in all rats to monitor reproductive cyclicity. After sacrifice the animals were examined for the presence of endometriotic lesions, and the colon was removed and examined for macroscopic damage. White blood cell (wbc) numbers in the peritoneal fluid were determined, and sections of colon were collected for measurement of myeloperoxidase activity (MPO). Segments of longitudinal and circular smooth muscle from colon and jejunum were mounted in tissue baths for isometric recording in response to acetylcholine (Ach; 10^{-9}-10^{-2}M). **Results:** Only experimental animals developed endometriotic cysts, no cysts were found in any of the sham-control or normal animals. Colonic damage and wbc number were significantly higher in experimental animals as compared to sham-controls (p<0.01) and normals (p<0.001). MPO levels were also highest in the experimental group. Longitudinal muscle from the colon of the experimental group exhibited significantly higher tension in response to Ach than that in either control or normal animals (p<0.05). This correlated with colonic damage and wbc numbers. There were no significant differences in either the longitudinal or circular muscle response of the jejunum between the groups, and no disruption of the estrous cycle. **Conclusions:** This study presents evidence for a significant effect of the endometriosis lesions growing intraperitoneally on both colonic function and integrity, which may help explain the gastrointestinal symptoms often associated with this disease. *This research was funded in part by SO6-GM08239 & NIH-RCMI 2G12RR03050.*

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Relationship between large bowel transit and abdominal pain in functional constipation

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Aim of this study was to evaluate the relationship, if any, between abdominal pain and the different patterns of large bowel transit, in functional constipation. Two hundred and thirty-one patients (yrs F:147; mean age: 38±15 yrs) seeking medical advice for functional constipation as defined with Rome II criteria[1] were interviewed by

means of a standardized questionnaire. Abdominal pain was referred by 175 pts (76%); subjects with and without abdominal pain did not differ for age (38±15 vs 38±16 yrs), the female/male ratio was significantly higher in the painful, than in the painless, group ($p<0.02$). Abdominal pain was considered severe by 20% of females and 3% of the males. All patients underwent gastrointestinal transit time using a previously published method[2]. Oro-anal transit time was abnormally prolonged in 88 pts (52%) of the painful constipation group. Pain was more frequently referred to the upper quadrants of the abdomen by pts with slow rectal transit (74%) and to the lower and lower-left quadrants by pts with slow colonic transit (68%) ($p<0.01$). **Conclusions** In patients with functional constipation abdominal pain: (a) is more frequent and more severe in females than in males; (b) was not related to delayed oro-anal transit time. Abdominal pain was more frequently referred to the upper abdominal quadrants by patients with slow rectal transit and to the lower and lower- left abdominal quadrants by those with slow colon transit.

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Effect of a non-specific muscarinic antagonist, tolterodine, on gastrointestinal and colonic transit in humans: a randomized, controlled study

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Background: While the non-specific muscarinic antagonist atropine inhibits GI motor activity and delays GI transit, the incidence of GI side effects with tolterodine, a non-specific antagonist used to treat urinary urgency and urge incontinence, is low. **Aim:** To evaluate the effect of tolterodine on GI transit in humans. **Methods:** In a parallel-group, double-blind study, 36 healthy subjects (24 women) stratified by gender were randomly assigned to tolterodine (4 mg LA) or placebo, for 6 days. Gastric emptying (GE), small intestinal (SBT), and colonic transit (CT) were assessed by scintigraphy on days 4–6. GE and SBT were measured by ^{99m}Tc-labeled egg meal. CT was measured by ^{111}In-labeled charcoal pellets within a capsule coated by methacrylate. Primary endpoints were GE $T_{1/2,}$ colonic filling at 6 h (CF_6, a measure of small intestinal transit), and geometric center (GC) of colonic transit at 24 h. Data were analyzed by an analysis of covariance; BMI, age, treatment, and interaction terms were covariates. **Results:** Thirty five of 36 subjects completed the study. Data were imputed for 1 subject who dropped out because of an acute illness unrelated to medication before the transit study. Tolterodine did not significantly affect GE, SBT or CT compared to placebo. GE and CT were longer ($p < 0.01$) in women. This gender effect was not modified by treatment. **Conclusion:** Tolterodine, administered at a dose widely used to treat urinary symptoms, did not significantly affect GE, SBT or CT in healthy subjects. Further studies are required to ascertain whether these observations can be explained by a balance between antagonism of muscarinic receptors which excite and which inhibit motility. Table show least squares, adjusted (for age, gender, BMI) means (95% CI). This research was funded in part by Pfizer Inc.

Parameter	Placebo (n = 18)	Tolterodine (n = 17)
Age	34 ± 2	33 ± 2
BMI (kg/m^2)	28 ± 1	27 ± 1
$GE_{T1/2,}$ min	116 (104, 127)	126 (112, 139)
CF_6 (%)	45 (33, 57)	36 (23, 48)
Colon GC 24	2.9 (2.5, 3.3)	2.6 (2.2, 3.0)
Colon GC 48	4.0 (3.6, 4.4)	3.9 (3.5, 4.3)
Median ascending colon $_{t1/2,h}$ (range)	12.0 (8.8, 15.2)	14.5 (10.9, 18.1)

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Hydrogen peroxide contributes to the impairment of Ca^{2+} release in colonic smooth muscle cells in colitis

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We have previously shown that neurokinin-A (NKA) is an important excitatory neurotransmitter in normal human sigmoid colon circular muscle and that NKA-induced contraction is mediated by release of calcium from intracellular stores. In ulcerative colitis (UC), however, NKA-induced Ca^{2+} increase and cell contraction of sigmoid circular smooth muscle were significantly decreased in calcium free medium, suggesting reduced Ca^{2+} release from intracellular stores. In the present study, we examined the role of hydrogen peroxide in the reduced Ca^{2+} signal in colitis. In UC sigmoid cicular muscle cells the decreased Ca^{2+} signal and contraction in response to NKA or thapsigargin were partly recovered in the presence of H_2O_2 scavenger catalase, suggesting involvement of H_2O_2 in UC-induced dysmotility. H_2O_2 levels were higher in UC than in normal sigmoid circular smooth muscle cells and enzymatically isolated UC muscle cells contained much higher levels of H_2O_2 than normal cells, which were significantly reduced by catalase. H_2O_2 treatment of normal sigmoid muscle cells in calcium free medium reproduced the reduction of NKA-induced Ca^{2+} release observed in UC cells. In addition, H_2O_2 caused a measurable direct release of Ca^{2+} from intracellular stores. In a model of dextran sulfate sodium (DSS)-induced colitis, the levels of H_2O_2 were significantly increased in the distal colonic circular muscle layer of wild-type mice, and NKA-induced contraction and intracellular Ca^{2+} increase were significantly decreased in circular muscle cells. In contrast, in catalase-overexpressing transgenic mice, production of H_2O_2 in distal circular muscle layer was significantly lower than in DSS-treated wild-type littermates and the reduction in NKA-induced cell shortening and Ca^{2+} signal in the distal colonic circular muscle cells was significantly lower than wild-type littermates. We conclude that H_2O_2 may contribute to the impairment of calcium release from intracellular Ca^{2+} stores in colitis.

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Role of the innate immune response in bacterially-induced muscle dysfunction

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Background: The impact of bacteria on enteric muscle function is poorly understood and the role of the innate immune response is unknown. We first determined whether bacterial infection produces persistent changes in muscle contractility, and next investigated whether the innate immune response induces alters contractility. **Methods:** C57Bl6 mice were infected with *C. rodentium*. Mice were sacrificed at 5, 7 and 9 weeks post-infection (PI). Sections of distal colon were stimulated with carbachol to measure muscle contractility. C57Bl6 and mCSF-1-deficient (*op/op*) mice received 50ug of CpG ODN (I.P.) in liposomes. Mice were sacrificed at day 2, 8 and 12 following CpG administration. Contractility was measured in the proximal jejunum. Disease severity and inflammation were evaluated by histology and myeloperoxidase activity (MPO). Cytokine analysis was determined using ELISA. **Results:** At week 6 PI, *C. rodentium* infected mice exhibit a 70% decrease in contractility, which began to resolve at week 8 PI and normalized by 10 weeks PI. Normal colonic MPO activity and increased mononuclear cell infiltrate were present at week 6 and 8 PI, indicating an absence of inflammation. Elevated colonic IFNγ present at week 8 resolved by week 10, corresponding with a reduction in mononuclear infiltrate and a normalization of muscle contractility. I.P. administration of a single dose of CpG in

liposomes induced a 60% attenuation in muscle contractility within 48 hours. This remained evident at day 8 and resolved by day 12. This was accompanied by an increase in MPO activity as well as a rapid and sustained increase in IL-23. An early transient increase in macrophages and polymorphonuclear cells was observed and was replaced by a mononuclear dominant low-level infiltrate with an increase in CD3+ T cells at day 8 and 12. These responses were absent in *op/op* mice. **Conclusion:** These finding indicate that bacterial infection is followed by persistent muscle dysfunction. The bacterial PAMP CpG induced persistent changes in muscle contraction mediated via macrophages. These results suggest that the innate response contributes to the induction of muscle dysfunction in the context of bacterial infection.

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Using combined impedance and manometry in the human ano-rectum to determine the site of origin of the defecating urge

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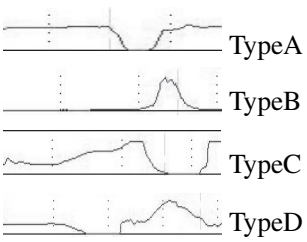
Rectal hyper or hyposensitivity, as determined through rectal balloon inflation, is implicated in various forms of constipation, yet none consistently so. A body of evidence suggests that under normal physiological conditions the rectum plays little or no role in the urge sensation. **Aims:** Determine proof of principle for use of impedance as a means of measuring stool/gas movement in the distal colon. 2. To identify the location of the receptors responsible for the defaecatory urge in the healthy colon. **Methods:** After an overnight fast 6 healthy subjects (4 female, 2 male) were given a distal colonic washout. With the aid of a flexi-sigmoidoscope, a combined high-resolution manometry (18 sideholes @ 3cm – spanning distal sigmoid colon, rectum and anal canal) and impedance (3 channels in the sigmoid colon @ 3 cm and 3 channels in the mid rectum to anal verge @ 3cm) catheter was positioned with the tip fixed in the sigmoid colon by endoclips. Subjects were given 4 durolax tablets to induce rectal filling. When subject perceived an urge to defaecate an event marker was pressed to record the time on the combined manometry/impedance trace. Onset of the urge was correlated with motor patters and changes in impedance at the various colonic regions. **Results:** The urge to defaecate was recorded in our 6 volunteers on 132 occasions. These urges were associated with a common cavity recorded throughout sigmoid, rectum and ano-rectum regions. Four different impedance wave types were associated with the urge sensation at each anatomical region (see fig). On 96% of occasions the urge was associated with Type B impedance change at the ano-rectal verge. 120 common cavities were also recorded in the absence of the urge sensation. These common cavities were of significantly reduced amplitude compared to those associated with an urge (15.8 ± 3.9 mmHg vs 24.7 ± 8.7 mmHg; p = 0.008) and they occurred in the absence of an impedance change at the ano-rectal verge. **Summary/Conclusions:** These data indicate; 1. Recording of impedance in the human distal colon is feasible; 2. The urge sensation may require stimulation of tension receptors at the anorectal verge secondary to the distal colonic common cavity.



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Purinergic and nitrergic neurotransmission in the colon of a transgenic (RIP/hIfnß) diabetic mouse

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Most of the gastrointestinal (GI) complications of long-standing diabetes relate to disturbances in GI motility. The underlying cause is thought to be multifactorial. Potential mechanisms include damage to the enteric nervous system (ENS) and to interstitial cells of Cajal (ICC). We have recently reported in the human colon a cotransmission between ATP and NO causing smooth muscle relaxation. Accordingly we have used a transgenic mouse (RIP/hIfnß) that reproduces human type 1 diabetes with very low doses of streptozotocin. **Aims:** 1- to investigate the spontaneous motility ''in vitro'' and purinergic-nitrergic cotransmission in colonic muscle strips from control (n=15) and diabetic (n=13) mice; 2- to correlate the functional data with structural data using immunohistochemistry (PGP 9.5, c-kit, nNOS and SP) on colonic whole mount preparations. **Results:** colonic circular muscle strips exhibit spontaneous contractions of about 1/min. The frequency of contractions was smaller in control compared to diabetic mice ($p<0.01$). Incubation of the tissue with TTX 1μM or L-NNA 1mM (but not MRS2179 20μM) increased its spontaneous motility ($p<0.001$) in control animals. In contrast, in diabetic animals the presence of this inhibitory neural tone was not observed or was reduced. In the presence of TTX the frequency and amplitude of spontaneous contractions was similar in both groups. EFS (60V, 5Hz, 0.4ms) inhibited the spontaneous motility in both groups. This inhibition was reduced by incubation with L-NNA and MRS2179. In the presence of both antagonists, an excitatory component was observed in diabetic but not in control animals. The immunohistochemical study of the myenteric plexus revealed no differences in the neural or ICC densities, or in the number of nNOs positive neurons between groups. However, SP-IR was higher in the diabetic animals. **Conclusions:** 1- a functional purinergic (through P2Y1 receptors), nitrergic neurotransmission causing smooth muscle relaxation is present in the mouse colon; 2- the inhibitory neural tone is mainly nitrergic but not purinergic; 3- despite the presence of a functional nitrergic-purinergic neurotransmission, an impairment of the inhibitory nitrergic tone is observed in diabetic animals; 4- an excitatory non-cholinergic component (possibly substance P) is present in diabetic but not in control animals; 5- colonic pacemaker seems unaffected in our model. The mouse colon is a suitable model to study the purinergic-nitrergic cotransmission recently described in humans.

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Impairment of colonic epithelial barrier function by acute stress in mice depends upon pancreatic trypsin secretion

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Background/Aims: Experimental acute stress induces a biphasic increase of colonic paracellular permeability (CPP) in mice. The late increase, observed three days after the stressful stimulus, markedly involves mast cells, T-cells and cytokine secretion. By contrast, the early increase that occurs 2-4 hours after stress initiation does not seem to be linked to a local immune system stimulation, and the underlying mechanisms are not known. The present study aimed at investigating the physiological pathways involved in the early increase of CPP induced by acute stress. Indeed, acute stress stimulates exocrine pancreatic secretion through cholinergic pathways, and trypsin is known to increase CPP through epithelial PAR2 activation. Consequently we have investigated in this work the possibility that trypsin released into the lumen following an acute stress may participate to the short-term increase in CPP. **Methods:** Animals (Swiss mice, 20–25 g) were treated i.p. (0.1 mL/mouse) with either atropine (5 mg/kg), or

a non selective CRF receptor antagonist (α-helical CRF (9–41), 1 mg/kg), or vehicle 30 min before being submitted to a 2h session of mixed restraint and acoustic stress. Then, CPP to 51Cr-EDTA and protease activity assay in colonic contents (total proteases and trypsin activity) have been determined. **Results:** Acute stress significantly increased CPP (1.9±0.1 vs. 1.1±0.1 %, P<0.05, n=8), total colonic proteolytic activity (3511±542 vs. 641±22 AU, P<0.05, n=6) and trypsin activity (213.5±44.5 vs. 83.3±18.3 AU, P<0.05, n=6). Atropine inhibited stress-induced impairment of CPP (1.3±0.1 %, P<0.05 vs. stress group, n=8), while the CRF receptor antagonist was devoid of effect. Similarly, atropine treatment strongly diminished total proteolytic and trypsin activity in stressed animals (1304±297 and 43.7±21 AU respectively, P<0.05 vs. stress group). No effect of atropine was observed in control animals. **Conclusions:** In conclusion, acute stress activates cholinergic pathways to trigger exocrine pancreatic secretion. Pancreatic enzymes, as trypsin, are responsible for colonic barrier alterations, likely through the activation of protease-activated receptors.

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GDNF modulates the increased neuronal oxidative stress associated with diabetic enteric neuropathy

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Background and Significance: Chronic diabetes causes complications such as gastroparesis and altered colonic motility which lead to significant morbidity. These motility related effects have been attributed to decreased intestinal inhibitory neuronal innervation. We have previously demonstrated hyperglycemia-induced apoptosis in enteric neurons, and the pathophysiological effects of hyperglycemia (apoptosis, loss of inhibitory neurons, motility changes) were reversed in diabetic GFAP-GDNF transgenic mice. We examined the role of oxidative stress in hyperglycemia-induced apoptosis of enteric neurons and the ability of Glial Derived Neurotrophic factor (GDNF), on modulating this oxidative stress. **Methods:** *In vitro* experiments were performed in primary enteric neurons isolated by immunoselection from digested E14 rat embryonic intestines. Apoptosis was assessed using the Hoechst and TUNEL method. Oxidative stress was measured by dichlorodihydrofluorescein (DCF) staining intensity and Glutathione peroxidase-1 (GPx1) mRNA expression. Diabetes was induced using intraperitoneal injection of streptozotocin in WT (WT-DM) and GFAP-GDNF overexpressing mice (GFAP-GDNF-DM). Control mice were injected with vehicle (WT-C and GFAP-GDNF-C). The GFAP-GDNF mice overexpress GDNF in glia under the GFAP promoter. *In vivo* oxidative stress was assessed by 8-OHdG and Glutathione peroxidase-1 (GPx1) expression. Laser capture microscopic dissection of myenteric ganglions from WT-C, WT-DM, GFAP-GDNF-C and GFAP-GDNF-DM mice was performed and mRNA obtained. This mRNA was used to assess the expression of GPx1 by RT-PCR. **Results:** Primary enteric neurons exposed to 20mM (24 h) glucose have a significantly higher level of apoptosis (TUNEL method) and levels of reactive oxygen species (DCF: 252 ± 2, n=3) compared to exposure to 5mM glucose (128 ± 3.2, P<0.001, n=3). Treatment of neurons cultured in 20mM glucose, with GDNF (100ng/ml) reduced the oxidative stress (177 ± 8.1, P <0.001 compared to 20mM glucose, n=3) and promoted survival. Eight weeks after the induction of diabetes in C57BL6 mice we find evidence of increased oxidative stress by 8OHdG staining in WT-DM animals compared to WT-C. GPx1 expression from mRNA isolated from WT-DM enteric neurons was higher compared to WT-C. GFAP-GDNF-DM mice had a reduction in GPx1 expression down to levels of WT-C or GFAP-GDNF-C mice. **Conclusions:** Hyperglycemia induces enteric neuronal cell apoptosis through increased oxidative stress. GDNF ameliorates these effects. These studies may provide novel therapeutic strategies to prevent the neuronal injury that occurs in patients with diabetes.

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Nociceptin/orphanin FQ- and nociceptin receptor- expression in patients with ulcerative colitis

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Background & Aims: Many stressors, such as mental stress, sometimes induce the aggravation of colitis in patients with ulcerative colitis. Nociceptin/orphanin FQ (Noc/oFQ), internal opioid-like peptide, is considered to relate with the neurogenic inflammation. We previously reported that the amelioration of DSS- induced colitis in Noc/oFQ receptor knockout mouse (Kato S et al. J Neuroimmunol. 2005, 161:21–8). However, the precise mechanism of Noc/oFQ in the ulcerative colitis is unclear. The aim of this study was to investigate the expression of Noc/oFQ and opioid receptor-like 1 (ORL-1) in human ulcerative colitis. **Methods:** The m RNA was extracted from the biopsy specimens of normal colon, and the inflamed and non-inflamed mucosa of ulcerative colitis (n=10). After reverse transcription, prepronociceptin mRNA was examined by real-time PCR. We also investigated immunohistochemistry against ORL-1, CD45RO (UCHL-1), CD20 (L26) and myeloperoxidase (MPO). **Results:** Prepro-nociceptin mRNA was significantly increased in the inflamed mucosa than both normal control and non-inflamed mucosa. ORL-1 expression was constitutively recognized in the small vessels of the submucosal layer of the colon. However, ORL-1 expression was increased in the small vessels and infiltrated cells of the lamina propria in the inflamed mucosa. ORL-1 was mainly expressed in the CD45RO- positive T lymphocytes and MPO- positive neutrophils, but not in the CD20-positive B lymphocytes. **Conclusion:** These results suggest that Noc/oFQ might aggravate colonic inflammation by the increase of migration of inflammatory cells, especially T lymphocytes and neutrophils via ORL-1.

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Evidence that Ca^{2+} release mechanisms are not involved in nitric oxide dependent post-junctional responses in gastrointestinal smooth muscles

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Stimulation of enteric inhibitory motor neurons leads to post-junctional membrane hyperpolarization known as an inhibitory junctional potential (IJP) in gastrointestinal muscles. In many regions IJPs consist of two components, a fast primary component mediated by purines and a slower, sustained component mediated by nitric oxide (NO). Recent reports have suggested that release of Ca^{2+} from intracellular stores may be necessary for nitrergic IJPs since this component was greatly reduced by caffeine, cyclopiazonic acid (CPA) and ryanodine. Our data have suggested that stretch dependent potassium channels (SDK) (possibly due to TREK-1) mediate nitrergic IJPs in the murine colon via a cGMP dependent mechanism. SDK and TREK-1 channels are Ca^{2+} independent channels, so we performed experiments to determine the role of Ca^{2+} release in nitrergic inhibitory responses in colonic tissues, colonic myocytes and transfected COS cells. NO-mediated IJPs in murine colon muscles were inhibited by caffeine (0.1- 10 mM) and CPA (1–10 μM), confirming the inhibitory effects of drugs that interfere with Ca^{2+}store release on nitrergic IJPs. However, we also found that IJPs were blocked by L-methionine (a blocker of TREK-1 channels). This suggested the hypothesis that caffeine and other store-active compounds may be inhibitors of native SDK channels and TREK-1. TREK-1 channels were stably transfected in COS cells. The expressed currents ranged from 2nA to 12nA in peak amplitude at +70 mV under dialysed whole cell configurations (external solution: MnPSS, internal solution: 10mM BAPTA with high K^+). TREK-1 conductances were inhibited by caffeine in a dose dependent manner (100 μM-5mM) with an IC_{50} of 1.4mM. TREK-1 currents were also blocked by the xanthine derivative, theophylline (1–5 mM), CPA (10 μM), thapsigargin (1μM)

and ryanodine (10 μM). SDK channels in native colonic myocytes were recorded from excised patches in which this conductance was maximally activated. Caffeine (5 mM) and L-methionine (1 mM) inhibited SDK channels. In summary, IJPs are inhibited by caffeine and CPA, but this effect is unlikely to be due to the function of these drugs on intracellular Ca^{2+} store release. Caffeine and CPA are inhibitors of TREK-1 channels, which appear to be responsible, in part, for the SDK conductance in GI muscles. Thus, the actions of store Ca^{2+} active drugs are likely to be due to the ability of these drugs to block SDK (TREK-1) channels in the GI tract. Supported by NIH P01 DK41315.

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Evaluation of digestive motility by Magnet Tracking System in a case of severe Hirschprung disease

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Hypoganglionosis, intestinal neuronal dysplasia (IND) are often found proximal to the transition zone (TZ) in patients with Hirschsprung's disease (HD). These abnormalities have been blamed for the bad outcome of surgery for HD. We describe an approach for analyzing the bowel motility that can help the surgical decision in patients with such questionable changes above TZ. **Case:** The patient was born at term after uneventful pregnancy. Since the 2nd day of life, she presented with absence of stool, abdominal distension and vomiting. Rectal biopsies were positive for aganglionosis. Laparotomies were performed for ileostomy and full thickness biopsies of the colon and terminal ileum, and for mapping the aganglionosis in the entire small intestine (SI). With 2 years of age, a Duhamel pull-through (PT) was decided. Our concern was addressed to the functional condition of the remaining SI. In order to assess its motility, the Magnet Tracking System (MTS)* was used before surgery and repeated one year after PT. Biopsies of SI and colon were evaluated by using hematoxylin-eosin and acetycholinesterase (AchE) stainings and standard histopathological criteria. For MTS, a magnetic pill (2.5x7mm) was placed in the stomach through a feeding tube during the 1st study and swallowed by the patient during the 2nd one. The trajectory of the pill was recorded with magnetic sensors incorporated into the bed. Biopsies revealed 40 cm of normal innervation distal to the duodeno-ujenunal flexure, 70 cm of dysganglionosis (IND, hypertrophied nerve fibers, hypoganglionosis or markedly increased AchE reaction, arranged in a mosaic form) and 26 cm of aganglionosis proximal to the ileocecal valve. Preoperative MTS showed normal gastric function (3.2±0.2 min^{-1} rhythm, 55 min sojourn), fast duodenal passage (100s, 8.2 $cm \cdot min^{-1}$) and 13–10 min^{-1} SI rhythm with no clear gradient but nevertheless very slow luminal progression. Two years after PT (100cm of SI left), the patient has normal bowel movements and excellent stool continence. The postoperative MTS showed normal gastric function (3.45±0.35 min^{-1}), SI transit of 4h with proximal and distal rhythms of 12.7 and 6–7.5 min^{-1}. In conclusion, MTS is an easily feasible and non invasive functional test. In our patient, it allowed to assess the bowel motility in areas previously described as pathological according to current histological criteria and prevented from repeating biopsies and resecting a long bowel segment. **Neurogastroenterol Motil. 2005;17:148.*

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Dynamics of human colonic transit as analyzed by Magnet Tracking System (MTS)

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The MTS allows to follow the movements of gastro-intestinal contents with high resolution in space and in time[1]. In this work we used the MTS to describe in some detail the topography and dynamics of colonic transit in young healthy subjects with regular daily habits. Nine males and 9 females swallowed a small magnetic pill on day 0 at 8h pm. The progression of the pill was recorded with magnetic field sensors placed over the abdomen and monitored on mornings 1 and 2 during 4 h sessions interrupted for breakfast (figure). The time spent in colonic segments was determined and phasic movements (mvmts) were analyzed with respect to their origin (table), amplitude, direction and speed.

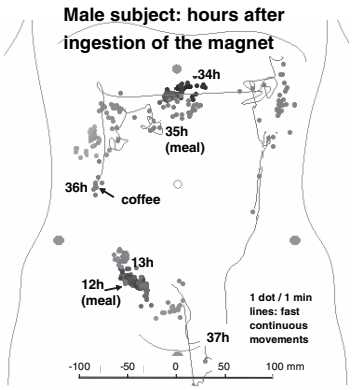
Oro-anal transit time was < 39h for 8 males and > 39h for 7 females. Two types of continuous movements (>10cm) were detected: slow ones (around 1cm/min) and fast ones (around 1cm/s). The fast movements were infrequent and mostly aboral, could cover several colonic segments, and occurred significantly more frequently in the left colon ($p<0.05$) and in males ($p<0.001$).

In conclusion, the MTS is well tolerated, reproduces many findings from different currently used clinical techniques and gives a rich information allowing to evaluate in detail the dynamics of segmental and total colonic transit.

[1]Neurogastroenterol Motil. 2005, 17: 148–154.

	caecum +ascend.	flex. hep. R transv.	L transv. f lex. spl.	descend.	sigma
recording time (h)*	35	16	17	10	18
total mvmts. F	9	10	8	9	9
total mvmts. M	9	11	24	26	17
aboral mvmts.	17	19	30	27	25
4-10cm	11	4	6	12	11
10-20cm	3	7	13	7	7
>20 cm	3	2	2	4	0
mvmts/h**	0.5	1.3	1.9	3.5	1.4

*same for F and M; **starting in, passing through or ending in



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Normal visceral pain reponses to colorectal distension in Nav1.9 knockout mice

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Tetrodotoxin-resistant voltage-gated sodium channels subtype 9 (Nav1.9) are expressed in small-diameter dorsal root ganglion neurons and have been involved in persistent somatic hyperalgesic responses associated to inflammation, but not in normal somatic pain responses (J Neurobiol 61:55, 2004; PNAS 102:9382, 2005). **Aim:** To assess the role of Nav1.9 channels on acute visceral pain responses associated to colonic mechanical stimulation in conscious mice, using Nav1.9 knockout (KO) animals. **Methods:** colorectal distension (CRD)-induced visceral pain was assessed in conscious wild-type and Nav1.9 KO female mice (C57BL/6 background). The mechanical activity of the abdominal muscles, assessed by measuring transient changes in

intracolonic balloon pressure during CRD (intracolonic manometry), was used as a measure of visceral pain (J Pain 7:108, 2006). Two CRD paradigms were used: phasic increasing (10–20–30–40–50–60 mmHg, 2 pulses of 10 s each, at 5 min intervals) or phasic repetitive distensions (55 mmHg x 12 pulses x 10 s each, at 5 min intervals). Data are mean±SEM. **Results:** CRD (10–60 mmHg) evoked abdominal contractions observed as reproducible CRD-related changes in intracolonic balloon pressure. Relative pain responses were similar in Nav1.9 KO (AUC for the mechanical response: 0.27±0.06, n=8) and wild-type mice (0.32±0.05, n=11; P>0.05 vs the response in KO mice). The threshold for pain, defined as the distending pressure eliciting changes in the mechanical activity of the abdominal muscles higher than the mean basal activity plus 3 times the standard deviation, was also similar in both groups (wild-type: 32.7±4.5 mmHg; Nav1.9 KO: 30.0±4.9 mmHg; P>0.05). Phasic repetitive CRD (55 mmHg) induced a sensitisation response, characterized by an increase in mechanical activity of the abdominal muscles over time. Sensitisation responses were similar in wild-type and Nav1.9 KO mice. When comparing pulses 10–12 with pulses 1–3, the mechanical response to CRD increased by 66±27 % and 107±35 % in wild-type (n=12) and Nav1.9 KO mice (n=8), respectively (P>0.05). **Conclusions:** These results suggest that Nav1.9 channels do not significantly contribute to visceral pain responses to acute colonic mechanical stimulation, as previously shown for normal somatic pain responses (PNAS 102: 9382, 2005). These observations, however, do not exclude a significant role of Nav1.9 channels in mediating persistent pain responses during hyperalgesic states.

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The motilin receptor agonist GM611 increases colonic motility via alterations in BK channel activity in the rabbit colon

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Motilin is a highly conserved 22-amino acid gastrointestinal polypeptide, which is involved in the regulation of GI motility. Its effects are mediated via the motilin receptor which belongs to the superfamily of G protein-coupled receptors. Upon its release, motilin acts to initiate Phase III of the indigestive migrating motor complex. It has previously been shown that erythromycin exhibits a motilin like acceleration of gastrointestinal emptying. To date several erythromycin derivatives have been developed eliminating the antibacterial actions of erythromycin whilst enhancing the motilin-like prokinetic activity. GM611 is a synthesized derivative of erythromycin that has increased acid stability and provides enhanced gastrointestinal motility, although the mechanism underlying this prokinetic activity is not fully understood. **Aim:** The aims of the present study were to elucidate the prokinetic mechanisms of GM611 using RT-PCR, standard isometric force measurements, sharp intracellular microelectrode recordings, and patch clamp recordings on rabbit colonic smooth muscle. **Results:** RT-PCR revealed the presence of motilin receptors in the rabbit colon. GM611 μM caused a 340% increase in the peak contractile amplitude of phasic contractions when compared to spontaneous phasic contractions prior to drug application. Mean tone also increased by 290%. Increases in the force and frequency of contractions were not affected by TTX suggesting a direct action of GM611 on smooth muscle. Intracellular microelectrode recordings revealed that GM611 caused a decrease in the interval between action potential complexes but did not cause a change in resting membrane depolarization or an increase in the amplitude of spikes. Patch clamp experiments on isolated smooth muscle cells revealed that GM611 had no effect on delayed rectifier K^+ currents and L-type Ca^{2+} currents from isolated rabbit colonic myocytes, but inhibited the amplitude of large conductance Ca^{2+}-activated K^+ currents. Rabbit colonic myocytes also demonstrated large spontaneous transient outward currents (STOCs). GM611 reduced the frequency but not the amplitudes of STOCs. **Conclusions:** These data suggest that the motilin receptor agonist GM611 increases GI motility through modulation of Ca^{2+} release from intracellular stores in rabbit colonic myocytes which leads to an indirect inhibition of BK channels. (supported by NIH P01 DK41315).

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CRF_2 receptor activation decreases colonic secreto-motor function in rats and Muc2 gene expression in DHE cells

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Background: CRF stimulates colonic transit and induces diarrhea through CRF_1 while inhibiting gastric emptying through the activation of CRF_2 receptors (Br J Pharmacol 8:1321-, 2004). CRF as well as stress cause mucus depletion from colonic goblet cells in rats (AJP, 271:G884-, 1996). Activation of CRF_2 mediates opposite effects to that mediated by CRF_1 in some endocrine and behavioral endpoints (Endocrinology, 144:2396-, 2001). **Aims:** Determine the effect of CRF_2 receptor activation on colonic motor and mucus secretion in rat as well as on mucin secretion and synthesis in DHE cells (Eur J Cell Biol 83: 347-, 2004). **Methods:** Adult male rats (280–320) received either ip saline (0.3 ml) or selective CRF_2 agonist, hUcn 2 (10 μg/kg) followed (+10 min) by ip saline (0.3 ml) or a non selective $CRF_{1/2}$ agonist, CRF (3 μg/kg). Fecal pellet output (FPO) was monitored for 60 min. Proximal and distal colonic tissue were processed for longitudinal muscle myenteric plexus (LMMP) whole mount CRF_2 immunohistochemistry and for histological analysis of goblet cells activity using alcian blue/periodic acid-Schiff (PAS)/hematoxylene stain. CRF_2 gene expression in DHE cells was determined using RT-PCR. DHE cell's mucin secretion response to Ucn 2 ($10^{-7,-8}$M) incubation was determined in the presence or not of bethanechol (10^{-3}M). **Results:** CRF, compared to saline, significantly increased FPO (0.0±0.0 vs 4.5±0.5/h). The CRF effect was blunted by pretreatment with hUcn 2 (2.0±0.3 v 4.5±0.5). CRF but not saline or hUcn 2 induced cavitation of mucus cells and decreased the number of detectable mucus cells/crypt (15.2 ± 0.5 or 18.3 ± 1.0 vs 10.5 ± 0.6). The CRF-induced cavitation and decreased mucus cell number is blocked by pretreatment with Ucn 2. Both colonic myenteric neurons and DHE cells express CRF_2. Incubation with Ucn2 did not affect DHE cells mucin secretion whereas bethanechol increased the basal secretion by over 90%. This effect was totally blocked by pre-incubation (–10 min) with Ucn 2. Ucn2 decreased also Muc2 gene expression in DHE cells. **Conclusion:** Peripheral CRF_2 receptor activation prevents CRF-induced colonic motor and mucin secretion in the rat colon and decreases mucin secretion and synthesis in DHE cells. CRF_2 may play a role in maintaining homeostasis during stress-induced secreto-motor alteration of the colon. Supported by DK57238 & DK68155.

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The relationship between intestinal transit and rectal sensorimotor function in patients with urge fecal incontinence

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Introduction: Patients with urge fecal incontinence (UFI) and rectal hypersensitivity (RH) have associated subjective increases in stool frequency and severity of urgency (Chan *et al.*, *DCR* 2005; **48**:134–40). This study aimed to determine whether accelerated colonic transit was contributory. **Methods:** A prospective study of 20 patients with UFI (median age 58, range 36–73, 14F), without organic disease, IBS, or psychopathology was undertaken. All patients underwent standardized clinical history and anorectal physiological investigation, including rectal compliance. Normal ranges from 56 healthy volunteers classified patients as having RH (n=9; median age 56, 36–68, 6F) or normal rectal sensation (NS: n=11; median age 62, 36–72, 6F). Both subgroups and 22 healthy control subjects (median age 32, range 21–72, 13F), underwent ^{111}In [DTPA] intestinal scintigraphy, with scans acquired

hourly from 1–11h, and at 24 and 26 h post oral isotope administration. The geometric centre of isotope mass (GCI) was calculated at 8 hours (when isotope was present in the colon of all controls and patients), and at 10, 11, 24, 26 hours. Percentage excreted at 24 h was also determined. **Results:** Patients with RH had greater stool frequency, and episodes of fecal urgency, compared to those with NS (P=0.03). The proportion of patients with attenuated resting/squeeze pressures was equivalent (P=1.0 & P=0.79, respectively), but rectal compliance was lower in the RH group (P=0.02). The progression of GCI at 8 hours was accelerated in the RH and NS groups (P <0.05), and at 10 and 11 hours for RH alone (P<0.05). Individually, 6 patients (RH = 3, NS = 3) had accelerated transit up to 11 h, and 3 (RH = 2, NS = 1) at 24 h. Despite increased stool frequency in the RH subgroup, the percentage of isotope evacuated at 24 h was similar to the NS subgroup (P=0.57), suggesting smaller volumes excreted at each bowel action. **Discussion:** This study has demonstrated accelerated intestinal transit in certain patients with UFI, but for the majority, with UFI and RH, stool frequency and urgency are unrelated to transit, and local sensorimotor mechanisms (rectal hypocompliance/hypersensitivity) may be more significant. However, increased transit may be important for some, and management of UFI, a condition traditionally attributed to anal sphincter dysfunction, requires comprehensive investigation, and when appropriate, treatments to normalize intestinal transit and modify rectal behavior. Further investigation is required to identify the site of accelerated transit when present (? upper gastrointestinal/ ? proximal colon).

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Rectal augmentation: short and mid term evaluation of a novel surgical procedure for severe fecal urgency and incontinence

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Introduction: A novel surgical procedure has been described (Williams *et al*., *DCR* 2001; **44**:192–8) for patients with fecal urgency and urge incontinence [UFI], associated with rectal hypersensitivity, low rectal compliance, and rectal hypercontractility. We report the short and mid term results. **Methods:** 12 patients (median age 42, range 32–56; 10F), with inability to suppress defecatory urge for more than seconds, underwent rectal augmentation, while 6 underwent simultaneous and 1 patient previous electrically-stimulated gracilis neosphincter (ESGN) construction. Clinical and symptomatic assessment by standardized questionnaires pre-operatively, was repeated at 1 and 5 years. Comprehensive anorectal physiological investigation, including rectal compliance and prolonged rectosigmoid manometry, were performed pre-operatively and at 1 year. **Results:** At one year, 1 of the 12 patients had a permanent stoma, being content with a loop ileostomy. By five years, 3 further patients (2 with ESGN) had permanent stomas constructed (subjective unsatisfactory reduction in urgency; worsening MS; severe new-onset rectal evacuatory difficulty). 9/11 patients (6 with ESGN) consented to one year follow up, while 5/8 patients consented at five years. Severity of fecal urgency decreased at one year, with ability to defer defecation improved from a few seconds to 10 minutes (P=0.0006), which was maintained at five years (P=0.028). Stool frequency fell from a median of 7 actions/day (range 4–15) preoperatively, to 2 (0.25–3) at one year (P=0.002), and 3.5 (1–8) at five years (P=0.028). Median Wexner continence score preoperatively was 15, which reduced to 3 at one year (P=0.005) and 4 at five years (P=0.027). For rectal function, maximum tolerated volume to balloon distension increased from 70 ml (range 26–90) to 125 ml (100–170; P=0.008), and rectal compliance increased from 7 ml/mmHg (3.5–8) to 14.8 ml/mmHg (10.3–18.4; P=0.008). Frequency of specific rectal contractile events (high amplitude contractions, rectal motor complexes) reduced, but not significantly. SF36 questionnaire results indicated mental health scores were unchanged, but physical health scores substantially improved at 1 (P=0.008), and at 5 years postoperatively (P=0.008). **Discussion:** For the majority, rectal augmentation ± neosphincter results in a significant, sustained symptomatic improvement in patients with fecal urgency and urge incontinence, reflected by improved physiological parameters when measured in the short-term. This supports the use of augmentation in highly selected patients.

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Serotonin transporter gene polymorphism and irritable bowel syndrome in the korean population

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Polymorphisms in the promoter region of the serotonin reuptake transporter (SERT) gene may underlie the disturbance in gut function in patients with irritable bowel syndrome (IBS). Association studies of SERT polymorphisms and IBS have shown diverse results among different countries, which might be due to racial and subject composition differences. The aim of this study was to assess the potential association between SERT polymorphisms and IBS in Koreans. A total of 190 IBS patients, who met the Rome II criteria, and 437 healthy controls were subjected to genotyping. SERT polymorphisms differed in the IBS and control groups (p = 0.014). The SERT deletion/deletion genotype occurred with greater frequency in the diarrhea-predominant IBS group than in the controls. A strong genotypic association was observed between the SERT deletion/deletion genotype and diarrhea-predominant IBS (p = 0.012). None of the clinical symptoms analyzed was significantly associated with the SERT genotypes. The frequency of the SERT insertion/insertion genotype was much lower than that of the other two genotypes. A significant association was observed between the SERT polymorphism and IBS, especially diarrhea-predominant IBS, suggesting that the SERT gene is a potential candidate gene involved in IBS in Korea.

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Analysis of rectal compliance in IBS patients using a non-linear model

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Background: The volume-pressure relationship in barostat test is often nonlinear. Therefore, a linear model may not detect differences in compliance between normal subjects and IBS patients. Our aim is to determine if IBS patients have abnormal rectal compliance using a non linear model. **Methods:** Fifty-nine IBS patients (aged 20–65 years; mean, 39.2 years; 31 women, 28 men) with symptoms which fulfilled Rome-II criteria and 21 healthy controls (aged 25–58 years; mean, 37.8 years; 11 women, 10 men) were recruited. Anorecatl functions including compliance were evaluated via barostat tests. And a power exponential model was used to fit the nonlinear compliance curve (by Bharucha et al. Vol = V_{max} x exp[-(κ x RelP)$^{\beta}$] with parameter β representing the overall shape of the curve, κ the change in balloon volume as a function of 1/pressure at any given point, $P_{1/2}$ the pressure at which half-maximum barostat volume is reached. And RelP relative pressure ($1/P - 1/P_{max}$)). **Results:** A linear function could not distinguish between the rectal compliances of normal subjects and IBS patients (10.3±3.1 and 8.9±2.9 mmHg, respectively. $P > 0.05$). However, even though no significant difference was detected in the overall shape of the curve (β), there were significant differences in κ and $P_{1/2}$ between normal subjects and IBS patients (Table 1). **Conclusion:** An exponential function provided the best fit to the actual data and can reveal altered biomechanical properties of the gut wall in IBS patients.

Table 1. Comparison of compliance curves between normal subjects and IBS patients

Parameter	Normal subjects	IBS patients	P
Kappa	13.3 ± 6.8	8.9 ± 5.5	0.01
Beta	0.66 ± 0.06	0.65 ± 0.12	0.83
$P_{1/2}$ (mmHg)	14.1 ± 4.5	9.37 ± 4.0	0.00

Values are presented as mean±SD.

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Sigmoid compliance and visceral perception in spinal cord injury patients

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Background and Aim: In traumatic complete or incomplete spinal cord injury (SCI) various continence mechanisms have been shown to be disturbed, resulting in either constipation or incontinence. Rectal or colonic compliance may be normal or reduced in SCI patients compared to controls. Theoretically, patients with cervical lesions, classified as having a complete injury according to clinical criteria (American Spinal Injury Association, ASIA), do not experience pelvic sensation. Nevertheless, some evidence reports a preservation of sensory functions in these patients. The origin of this sensation has not been determined yet and data on physiological features of sigmoid compliance and perception in SCI patients are lacking. The scope of this study was to investigate sigmoid compliance and perception in chronic SCI patients. **Methods:** We evaluated sigmoid responses to fixed tension distensions applied by means of a tensostat in 6 patients (6M, 42±4 yrs) with complete transsection of the spinal cord (5 tetraplegic C5-C7 and 1 paraplegic T4-T6) classified as ASIA-A and impaired evacuation (i.e., constipation). Results were compared to those obtained in 10 healthy subjects (6M, 25±1 yrs). **Results:** SCI patients had higher sigmoid compliance at the highest distension level than controls (10.3±2.4 mL/mmHg vs. 5.1±0.8 mL/mmHg; $P<0.05$) and reduced maximal tension tested (54±14 g vs 85±4 g; $P<0.05$). Perception scores at first sensation were higher in patients (2.3±0.7 vs. 1.1±0.1; $P<0.05$), but were not different at the highest distension levels (3.7±0.8 vs. 3±1; NS). In 2 patients, sigmoid distension induced autonomic dysreflexia. Compared to controls, the most commonly reported sensation by patients was distension/bloating (70% vs. 54%, respectively; NS). Patients reported sensations less commonly to the hypogastrium (the sub-umbelical region) compared to controls (29% and 88%; NS). **Conclusion:** Patients with ASIA-A SCI have: 1) increased sigmoid compliance; 2) maintenance of visceral perception, which appears differently referred from that defined by controls. These data indicate that increased compliance is an underlying mechanism of abnormal evacuation in these patients. The preservation of visceral sensations, although abnormally referred, implies the occurrence of sensory input remodelling at spinal level.

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Relationship between AH neuron excitability and peristalsis in normal versus inflamed guinea pig distal colon

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Functional abnormalities in the colon that accompany inflammatory bowel disease likely involve changes in physiological properties of enteric neurons. We have recently used the TNBS model of colitis to investigate the effects of inflammation on neural circuitry in the guinea pig distal colon. From electrophysiological studies we have seen that AH (sensory) neurons fire more action potentials, have smaller afterhyperpolarizations, and exhibit increased spontaneous activity. The afterhyperpolarization of AH neurons is initiated by an inermediate conductance, Ca^{2+}-activated K^+ (I_K) current (blocked by TRAM34) and terminated by a hyperpolarization-activated I_H current (blocked by ZD7288). An increase in this I_H conductance appears to be responsible for the attenuated afterhyperpolarization and increased excitability in colitis. We have also observed disrupted peristalsis in the inflamed colon, therefore we reasoned if hyperexcitability of AH neurons plays a role in dysmotility, then TRAM34 should reduce motility in normal colon, while ZD7288 should restore motility in inflamed colon. The purpose then of this study was to further characterize the dysmotility in TNBS-inflamed colon and to test the hypothesis that excitability of AH neurons affects the rate of peristalsis. We recorded rates of pellet propulsion and generated spatiotemporal maps of normal and inflamed colons using the MedAssociates Gastrointestinal Motility Monitor system. Our results show that in normal tissue the velocity of pellet propulsion was 1.92 ± 0.08 mm/sec (N=57). For inflamed tissue, velocity was comparable to control in non-ulcerated regions (2.08 ± 0.11 mm/sec, N=22), but was disrupted at ulcerated sites. In normal colon, the I_K blocker TRAM34 (10 μM) reduced velocity (Krebs, 2.20 ± 0.17 mm/sec; TRAM34, 1.59 ± 0.13; $p\leq 0.005$, N=16), whereas the I_H blocker ZD7288 (10 μM) increased velocity (Krebs, 2.090 ± 0.24 mm/sec; ZD7288, 2.393 ± 0.29; $p\leq 0.05$, n=16). In inflamed tissue, ZD7288 did not alter velocity in non-ulcerated regions, but appeared to facilitate movement of pellets through ulcerated sites. In summary, factors that alter the afterhyperpolarization, and thereby excitability, of AH neurons do appear to affect motility of the distal colon. Though the means by which they do so are not straight-forward, as blockade of I_K or I_H channels in normal tissue reduces or increases propulsion respectively, whereas blockade of I_K conductance in inflamed tissue appears only to affect ulcerated sites.

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Luminally released serotonin (5-HT) stimulates colonic motility and accelerates colonic transit in rats

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Background Serotonin (5-HT) plays an important role in regulation of gastrointestinal (GI) motility. 5-HT is released from enterochromaffin (EC) ells into the portal circulation or basolateral border of the mucosa (J Clin Gastroenterol 2005;39:S184-S193), while others showed that 5-HT is released into the lumen of GI tract (Science 1981;213:1254–1255, Histochem Cell Biol 1997;108:105–113). The physiological role of released 5-HT into the colonic lumen is poorly understood. We hypothesize that luminally released 5-HT from EC cells of the proximal colon is transferred distally with feces and stimulates motility of the distal colon, resulting in acceleration of colonic transit in rats. **Methods:** Content of 5-HT in the colonic mucosa and feces were measured by HPLC. 5-HT content in feces of the proximal colon was also measured before and after the restraint stress-loading. To investigate whether luminal 5-HT is involved in regulating colonic motility, the distal colonic motility was recorded under the stress-loading and a $5\text{-}HT_3$ receptor antagonist (ondansetron, 10^{-5} M, 0.5 ml; 1.8 μg/rat) was administered intraluminally of the distal colon in conscious rats. To obtain the direct evidence that 5-HT is released into the lumen, we utilized *ex vivo* model of isolated vascularly and luminally perfused rat proximal colon. Luminal and vascular perfusate was collected every 3 min for 5-HT assay. To investigate whether intraluminal 5-HT accelerates colonic transit, 5-HT and ^{51}Cr (a marker for colonic transit) were administered into the lumen of the proximal colon. **Results:** Tissue content of 5-HT in the proximal colon (15.2±4.3 ng/mg wet tissue, n=5) was significantly higher than that in the distal colon (3.3±0.7 ng/mg wet tissue, n=5, P<0.01), while fecal content of 5-HT was almost the same in the proximal (790±168 pg/mg wet feces, n=6) and distal colon (796±146 pg/mg wet feces, n=6). Restraint stress significantly increased the fecal content of 5-HT in the proximal colon (1,112±165 pg/mg wet feces, n=6), compared to controls (694±95 pg/mg wet feces, n=7). Elevation of intraluminal pressure (10 cmH_2O) increased the luminal release of 5-HT (974±300% increase of basal, n= 5), but not vascular release of 5-HT (141±29% increase of basal, n= 5). Restrain stress-induced stimulation of the distal colonic motility was significantly attenuated by the luminal administration of ondansetron. Luminal administration of 5-HT (10^{-6}-10^{-5}M; 0.5 ml) significantly accelerated colonic transit. **Conclusions:** It is suggested that the elevation of intraluminal pressure stimulates 5-HT release into the lumen, not the portal circulation, of the rat proximal colon. Luminally released 5-HT from the proximal colon may have an important role to regulate the distal colonic motility.

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Abnormalities of the enteric nervous system, smooth muscle and interstitial cells of Cajal in children with colonic motility disorders

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Objectives: Abnormal colonic motility patterns are generally classified as myopathic or neuropathic. The current study evaluates the relationship between colonic manometry findings and changes of the colonic enteric nervous system (ENS), smooth muscle and interstitial cells of Cajal (ICC). **Methods:** Colonic specimens from surgical resections and full-thickness biopsies were obtained from 12 children (4 male), median age 4.5 (range 1–18) who underwent prior colonic manometry testing. Eight children had severe constipation of unknown origin, and 4 had Hirschsprung's disease. Colonic motility was considered normal when High Amplitude Propagated Contractions (HAPC) occurred in the evaluated segment. Low Amplitude Propagating Contractions (LAPC) and absence of contractions (AC) were considered suggestive of myopathic changes and simultaneous contractions (SC) were considered suggestive of neuropathic changes. Immunohistochemistry stains were used to identify abnormalities of the ENS: neuron specific enolase, calretinin, neurofilament, synaptophysin (neuronal markers), tyrosine hydroxylase (adrenergic pathway marker), S-100 (Schwannian derivation), GFAP (glial fibrillary acidic protein). ICC were stained with CD117 (c-kit), and smooth muscle markers included SMA (alpha actin) and MSA (gamma actin). **Results:** Of 25 ganglionic colonic segments, taken from different colonic regions, both motility and immunohistochemistry were evaluated (table). **Conclusions:** Although a myogenic motility pattern was observed, no muscle abnormalities were found using actin markers. Loss or absence of ICC and elevated levels in GFAP were prevalent findings in pediatric colonic motility disorders, irrespective of the manometric pattern. Caution should be used when predicting the type of underlying neuromuscular disorder based on colonic manometry.

Immunohistochemistry findings	HAPC (n=9)	LAPC (n=3)	AC (n=3)	SC (n=10)
Ganglia abnormalities (S-100) – n (%)	1 (11)	2 (67)	0	3 (30)
Gliosis (GFAP) – n (%)	4 (44)	1 (33)	2 (67)	6 (60)
ICC, absent or rare – n (%)	6 (67)	3 (100)	3 (100)	9 (90)
Muscle actin abnormalities – n	0	0	0	0

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Reproducibility of assessment of rectal sensory and motor function using two different barostat systems

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Background: Barostat studies have been used extensively to study visceral sensori-motor function in man. Currently, 2 types of devices are commonly used: the dual stage rigid cylinder type (capacity 100 ml; Synectics) and the single stage matched reservoir type (capacity 1200 ml; G&J). The former has the theoretical disadvantage of error due to non pre-pressurising of that cylinder excluded from the circuit. Comparing the two systems *in vivo* determines the validity of comparisons of data, especially contemporary vs. historical, within retrospective comparisons, multicentre studies, and meta-analysis of literature. **Methods:** Four rectal barostat studies were performed on 7 healthy volunteers (3F, 4M; median age 46), using each of the 2 barostat systems (dual stage [system A] and single staged matched reservoir [system B]) randomly twice (on two consecutive days, repeated two weeks apart). After conditioning distension, and determination of the operating pressure (minimum distending pressure+2 mmHg), an ascending method of limits phasic distension protocol was used, with 4 mmHg increments up to maximum tolerated pressure. Between phasic distensions, baseline pressures were maintained at operating pressure, and volumes (residual volume) recorded. Pressures and volumes at different sensory thresholds (first sensation, defaecatory desire, maximum toleration), as well as residual volumes, were compared between the two systems within individuals. Compliance curves were also assessed. **Results:** Operating pressures (mean operating pressures [± SEM]: (**A**: 6.4 ± 0.2 mmHg, **B**: 6.7 ± 0.2 mmHg, P=0.84, paired t test) and compliance (**A**: 7.7±0.6 mls/mmHg, **B**: 7.9 ±0.6 mls/mmHg, P= 0.42, paired t test) within subjects were equivalent; Bland-Altman statistic showed a mean difference in compliance of −0.2 mls/mmHg (95% limit of agreement −2.3 to 1.9). Similarly, pressures and volumes at sensory thresholds were similar within subjects using the two barostat systems (P >0.05, repeated measures ANOVA). Residual volumes at operating pressures were also similar: **A**: 104±16 mls, **B**: 97±15 mls (P>0.05, paired t-test). **Conclusion:** *In vivo* assessment of rectal biomechanical properties was equivalent when determined by the two most commonly used barostat systems. At least with regard to the ascending method of limits, studies performed using these different systems are comparable.

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Does anticipation influence rectal sensori-motor parameters in healthy volunteers?

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Background: The barostat has been used extensively to evaluate visceral sensori-motor function in man. Good reproducibility has been observed in most studies performed in healthy volunteers. Fear and anticipation may have an impact on the results of rectal sensory testing, particularly when assessed by procedures which are invasive, and in which the stimulus can be a source of discomfort. The aim of this study was to assess the impact of different time intervals between repeated rectal barostat studies on sensory thresholds and ratings in healthy volunteers. **Methods:** Rectal barostat studies were performed on 7 healthy subjects (3F, 4M; median age 46) on 4 occasions (two consecutive days, repeated two weeks apart). After conditioning distension, and determination of the operating pressure (minimum distending pressure [MDP] +2 mmHg), an ascending method of limits phasic distension protocol was used, with 4 mmHg increments up to maximum tolerated pressure. Between phasic distensions, baseline pressures were maintained at operating pressure. After a rest period of 15 minutes, rectal sensory ratings (VAS) for urgency to three random phasic distensions (MDP+12, +22 and +32 mmHg), lasting 1 minute with a rest period of 1 minute between distensions, were also determined. Pressures and volumes at different sensory thresholds (first sensation, defaecatory desire, maximum toleration), as well as sensory ratings were compared between consecutive days and between days 2 weeks apart in the same individual. Compliance curves were also assessed. **Results:** Operating pressures (mmHg) and compliance (mls/mmHg) within subjects showed no significant difference between consecutive days (mean operating pressures [± SEM]: 6.6±0.2 vs. 6.7±0.2,P =0.87); compliance: 7.7±0.6 vs. 7.9±0.6; P=0.50), or between days 2 weeks apart (operating pressures: 6.5±0.2 vs. 6.7±0.2, P= 0.50; compliance: 7.6±0.5 vs. 7.9±0.6; P=0.37). Pressurse and volumes at sensory thresholds were also similar within subjects on consecutive days and on days 2 weeks apart (P>0.5 repeated measures ANOVA). In addition, rectal sensory ratings to random phasic distensions were equivalent on consecutive days (MDP+12: 1.4±0.4 vs. 1.4±0.4; MDP+22: 3.6±0.7 vs 3.7±0.5; MDP +32: 5.3±0.7 vs. 5.4±0.8), and when measured 2 weeks apart (MDP+12: 1.2±0.3 vs. 1.7±0.4; MDP+22: 3.5±0.6 vs 3.8±0.7; MDP+32: 4.8±0.8 vs. 5.9±0.7). **Conclusion:** Assessment of rectal sensory function and wall mechanics in healthy volunteers is reproducible and independent of time interval between studies. Whether such reproducibility exists within patient groups is yet to be determined.

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Is the rectal contractile response to distension altered in patients with rectal hyposensitivity?

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Background: Rectal hyposensitivity (RH) relates to diminished rectal sensory perception, and is commonly found in patients with constipation, faecal incontinence or both (Gladman *et al., DCR 2003*;**46**;238–46). The rectal contractile response (RCR) to rapid phasic distension has been demonstrated previously (Akervall *et al., Gut* 1989;**30**,496–502); its significance in healthy individuals is not fully understood, although an exaggerated RCR has been implicated in the pathophysiology of patients with IBS (Corsetti *et al., Clin Gastroenterol Hepatol* 2004;**2**:49–56). **Aims:** To assess parameters of the RCR to phasic distension in patients with constipation, and to assess the impact of rectal sensory function. **Methods:** The study cohort comprised 31 patients (25 F; median age 47, range 23–71) referred for investigation of symptoms of chronic constipation. The patients were classified on the basis of elevation of sensory thresholds to volumetric balloon distension into RH (n=21) and normal sensation (NS: n=10). All patients and 10 healthy volunteers (HV: 7 F; median age 40, range 25–58) then underwent an electromechanical barostat study to assess rectal compliance, by which the RH group was further categorized into those with abnormal compliance (n=10) and those with normal compliance (n=11). All patients with NS had normal compliance. Furthermore, parameters of the RCR to phasic isobaric distension at minimum distending pressure+4 mmHg were analyzed; at this pressure threshold, the maximum RCR amplitude (reduction in volume) was observed in healthy volunteers. For each RCR, reduction in volume, and area over the curve (AOC) were calculated as percentages of the total volume and total area for the duration of the response, respectively. In patients, values below mean-2SD (reduction in volume <21.4%; AOC <9.2%), derived from the HV data, were considered abnormal. **Results:** The percentage reduction in volume, and AOC were significantly reduced in 13/21 patients with RH, compared to only 1/10 patients with NS (P=0.008, Fisher's exact test). In those RH patients with rectal hypercompliance, 7/10 had a blunted response; in those with normal compliance, 6/11 had a blunted response (P=0.66). **Conclusion:** The rectal contractile response to phasic distension is diminished in patients with rectal hyposensitivity. This may be independent of rectal wall properties. The pathoaetiology and clinical significance of the RCR in these patients warrants further investigation.

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Evaluation of somatic nerve function in patients with rectal hyposensitivity

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Background: Rectal hyposensitivity (RH) relates to diminished rectal sensory perception, and has been commonly observed in patients with constipation, faecal incontinence or both (Gladman *et al., DCR* 2003; 46; 238–46). RH has also been found in association with bladder hyposensitivity (Gladman *et al., NGM* 2004; **16**; 831), suggesting a pan-pelvic autonomic neuropathy. However, it is unclear whether this reflects a more proximal neurological lesion, or more generalized neuropathy. This study aimed to systematically evaluate general and sacral somatic sensory function in constipated patients with rectal hyposensitivity. **Methods:** Eighteen patients with constipation (13 F; median age 46, range 27–62) and 19 healthy volunteers (HV:10 F; median age 46, range 32–55) were studied. Patients were divided into normal rectal sensation (NS: n=8) and RH (n=10), based on elevation of sensory thresholds to balloon distension and rectal mucosal electrostimulation above the normal ranges for our unit. No patient had a history of overt neurological disease, or of conditions associated with neuropathy (e.g. DM, MS). Thresholds for cool and warm sensation were measured using a thermal stimulator (Thermotest, Sweden) and the sums of the differences were calculated. The average of the differences in thresholds over five stimuli was taken. The S2, 3 & 4 dermatomes in the perineum were assessed bilaterally and the C6 (hand), and L5/S1 (instep of foot) were studied for comparison. Sural and peroneal nerve conduction velocities were also determined to exclude generalized somatic neuropathy. **Results:** Sensory thresholds to thermal stimulation were significantly abnormal in the perineal dermatomes in patients with RH compared to NS and HV (RH vs. NS: P<0.01; NV vs. RH: P<0.01: NV vs. NS: P >0.05). In the RH subgroup, of the 20 thresholds tested, 6 were abnormal in 4 patients; 0 of 16 thresholds were abnormal in the NS subgroup (P=0.05; Fisher's exact test). Thermal sensory thresholds were similar in the RH and NS subgroup in the hand and the foot (P=0.47, and P=0.21, respectively), and all were within the normal range. Peroneal and sural nerve conduction velocities were similar in all three groups (P>0.05), and no individual values were abnormal in the RH or NS subgroups. **Conclusion**: Somatic nerve function is normal in the majority of patients with rectal hyposensitivity. However, in 40% of RH patients studied, there is evidence of a specific sacral somatic sensory neuropathy, suggesting an isolated pelvic neuropathy, and indicating involvement at an anatomical level distal to or including the sacral roots.

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Effect of motiltin agonist on colon motility in children

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Introduction: Motilin has excitatroy effects on the colon of rabit and the dog, but little is known of its effect on the human colon. Motilin and its aganoist erythromycin were shown to have a direct excitatory effect on the circular smooth muscle from human colon, which was mediated through the motilin receptor. It is well known that plasma motilin concentration fluctuates with changes in the phase of the migrating motor complex (MMC) in the stomach and the small bowel. Exogenous motilin induces phase III of the MMC in the small bowel. **Aim:** To evaluate the effect of intravenous erythromycin on colon contarctions using colon manomatry (CM) in children. **Methods:** We did a retrospective evaluation of 11 children who had normal and simultaneously recorded ADM and CM studies. Erythromycin was given intravenously (1mg/kg). All subjects had normal gastric antrum contractions and phase III of the MMC after intravenous erythromycin. After removing the artifact we claculated area under the curve (AUC) for 1 hour of fasting and post erythromycin phases of the CM study. The motiltiy recordings were also evaluated for high amplitude propagating contarctions defined as colon contraction at least 60 mmHg in amplitude and propagating over at least 30 cm of colon. Data analysis was performed using SPSS software, version 10.1 for Windows. We used students t test to compare the AUC. **Results:** The mean age was 9.6 yrs (range 4–12 yrs) and there were four males. All patients had normal ADM with gastric antrum contractions and phase 3 like activity following intravenous erythromycin. The mean (SE of mean) AUC for fasting phase of the CM study was 2.1 mmHg/sec (0.35) compared to 0.99 mmHg/sec (0.17) for the post-erythromycin phases of the study. The post erythromycin AUC was significantly less than fasting AUC, p value <0.01. Only one patient had a HAPC following erythromycin. All patients had HAPCs after bisacodyl administered directly into the colon through the manometry catheter and the mean duration of onset of bisacodyl effect was 7.18 minutes (range 1–10 minutes) and the mean number of HAPCs was 8.9 (range 1–16). **Conclusion:** Intravenous erythromycin in a dose sufficient to induces gastric antrum contractions and phase III of the MMC in small bowel does not stimulate colon contractions.

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Distribution and localization of corticotropin-releasing factor (CRF) receptor 1 mRNA in human GI tract and its expression in the mucosa of jejunum and colon in patients with IBS

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CRF signaling pathways are responsible for mediating the gut response to stress via binding to two distinct receptor types, CRF_1 and CRF_2. However, most of this knowledge is derived from animal studies. In humans, the distribution of CRF_1 within the whole GI tract is unclear. CRF IV increased colonic motility and IBS patients had a greater response compared to healthy subjects (HS). Aims of this study are to map CRF_1 mRNA distribution in human GI tract, examine the location of CRF_1 in the colon and compare the expression of CRF_1 mRNA in jejunal and colonic biopsies of HS and IBS patients. The whole thickness tissues of GI tract from 4 male HS (24–29 yrs) were processed for RT-PCR using human CRF_1 primers. CRF_1 immunohistochemistry was performed on both whole thickness tissue and the colonic biopsies of HS. 14 jejunal biopsies from HS (3 females, F, 3 males, M) and diarrhea-IBS patients (IBS-D) (4 F, 4 M), 32 sigmoid colonic biopsies from 8 HS (4 F, 4 M) and 24 IBS patients with predominantly constipation (IBS-C) or alternating (IBS-A) (16 F, 8 M) were processed for real-time PCR. RT-PCR showed that CRF_1 mRNA was present in each investigated segment. The highest level was observed in the ileum and rectum, followed by jejunum, duodenum, esophagus, cecum, pylorus, gastric fundus and colon. In the sigmoid colon, CRF_1 immunoreactivity was mainly located in the lamina propria, submucosal and myenteric plexus. Real time PCR showed that CRF_1 mRNA level in the jejunal mucosa of HS is significantly higher in male than in female. CRF_1 mRNA levels tend to be increased in both jejunal (38%) and colonic mucosa (8%) in female IBS patients compared to HS. In contrast, a decreased tendency was found in both jejunal (23%) and colonic mucosa (19%) in male IBS patients. No significant difference in CRF_1 mRNA levels in the colonic mucosa was seen between IBS-C and IBS-A. These results indicate that CRF_1 mRNA is expressed in whole GI tract in humans with different levels in distinct segments. CRF_1 is located in the colonic lamina propria, submucosal and myenteric plexus. There is a sex-based difference between the HS and IBS in jejunum and colon. Supported by NIH grant P50 DK64539 & FIS grant CM04/00019.

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Scintigraphic pattern of colonic transit does not differentiate between patients with isolated slow transit constipation (STC) and those with coexistent rectal evacuatory disorder (RED)

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Background: Patients with severe constipation may be subclassified on the basis of speed of colonic transit and efficacy of rectal evacuation. Colonic scintigraphy is able to define patterns of transit and defaecating proctography yields information on the nature of disordered rectal evacuation. It has been postulated that a severe RED may be associated with a secondary transit delay that is predominantly left-sided. The aim of this study was to determine whether scintigraphy can discriminate between patients with STC with or without coexistent severe RED on the basis of progression of isotope throughout the colon and by analysis of specific regions of interest. **Methods:** 196 patients with STC diagnosed on the basis of a radio-opaque marker study were sub-classified according to results of proctography and rectal sensory testing. Severe RED was defined as ≥2 abnormalities on proctography (high % retained neo-stool, delay in evacuation and mechanical obstruction to outflow) and/or rectal hyposensitivity; absence of RED was defined when no abnormality was observed on either test. These 2 divergent STC subgroups, those with severe RED and those without any RED, subsequently underwent ^{111}In[DTPA] colonic scintigraphy. Percentage activity in 7 defined regions of interest (6 colonic, 1 for excreted stool) were determined, and time-activity curves generated using the geometric centre of isotope mass (GCI). Patterns of colonic transit were classified (by convention) as generalized (GCI <3.6 at 48 h), or distal (GCI >3.6 at 48 h). **Results:** 44 patients had STC only (STC only-group), and 35 subjects had STC with defined severe RED (RED-STC group: neo-stool excreted 31.0 ± 4%, time for evacuation 241 ± 11 sec; maximum tolerable volume: 300 ± 21 ml). Time activity curves and patterns of delay were similar between the 2 groups (left sided delay: 21/44 STC only-group vs. 17/35 in the RED-STC group; Fisher's exact test, P=1.00). Similarly, no significant differences were observed between groups in percentage elimination of isotope from the right colon at 18 hours, and activity in the left colon at 72 hours. **Conclusion:** Colonic scintigraphy failed to discriminate between patients with STC with or without coexistent severe RED, with no differences in generalized or regional transit being evident. These data support the view that RED is *not* associated with a specific pattern of transit delay and that scintigraphy alone cannot predict the presence or absence of RED, knowledge of which is important to management. The influence of RED on colonic transit (e.g. by reflex activity) requires further study.

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Neurotoxins may diminish gastrointestinal motility by peripheral action

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Background: Gastrointestinal (GI) dysmotility after neurotoxins intake has been attributed in part to their peripheral action on GI tract, however the direct neurotoxins effects on GI reflex activity and structure of the interstitial cells of Cajal (ICC) remain unexplained. Aim: Evaluation of the effect of chronic salsolinol administration, the neurotoxin involved in Parkinson's disease, on duodenal myoelectrical activity (DMA), vagal afferent activity (VAA) and duodenal c-Kit expression. **Methods:** Ten rats were receiving intraperitoneal injections of salsolinol (50 mg/kg/day) for 3 weeks, whereas the equal group served as control. In both groups frequency of DMA and VAA potentials were analysed (ADInstrument, Australia) during fasting and stepwise gastric distension (GD) of 10 ml. Subsequently duodenal fragments were removed and intramuscular ICC were visualised using the rabbit polyclonal anti-c-Kit antibodies (Santa Cruz Biotechnology, USA) and the LSAB/HRP (DAKO Corporation, USA) dye set. **Results:** Analyses of the fasting DMA and VAA recordings didn't reveal differences between the compared groups. During GD DMA frequency remained unchanged in the controls whereas in the salsolinol group slightly decreased (p=0.04). Simultaneously increase of VAA frequency due to GD was observed in the control (p<0.01) but not neurotoxin group. Image analysis revealed lower c-Kit expression in the duodenum of the salsolinol-injected animals (p=0.05). **Conclusions:** Our study revealed decreased c-Kit expression and suppressed DMA response to GD with lack of VAA reaction to GD in the salsolinol-injected rats. Those results may suggest the direct effect of the neurotoxine on both ICC and neuronal pathways of vagal and gastro-duodenal reflexes.

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Neuropathological aspects of the colon in obstructed defecation

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One of the most frequent subtypes of constipation is represented by obstructed defecation, and it has recently been reported that these patients may have colonic motor abnormalities in addition to altera-

tions of the anorectal area. However, it is unknown whether these patients display abnormalities of the enteric nervous system, as reported in other groups of constipated subjects.

We evaluated the neuropathologic aspects of the enteric nervous system in a homogeneous group of patients with obstructed defecation. Colonic specimens from 11 patients (nine women, age range 39–66 yr) undergoing surgery for symptoms refractory to any therapeutic measure, including biofeedback training, were obtained and examined by means of conventional histological methods and immunohistochemistry (NSE, S100, CD117, CD34, Bcl-2, formamide-Mab). Control specimens were obtained from patients that underwent surgery for nonobstructive colon cancer.

The enteric neurons were significantly decreased only in the submucosal plexus of patients ($p<0.0001$ vs controls), whereas the enteric glial cells of constipated patients were reduced in both the myenteric ($p= 0.018$ vs controls) and the submucosal plexus ($p= 0.004$ vs controls). No difference between patients and controls were found concerning c-Kit and CD34 expression, and the number of apoptotic neurons.

We conclude that, in at least a subgroup of patients with obstructed defecation and severe, intractable symptoms, abnormalities of the enteric nervous system, mostly related to the enteric glial cells, are present. Since the enteric glial cells are involved in multiple aspects of gastrointestinal motility, from mechanical support to neurotransmission, these findings might explain some of the pathophysiological abnormalities. Moreover, such studies could help to better understand this condition, which usually responds to biofeedback treatment , to explain the lack of such response and, possibly, to aid in developing more targeted therapeutic approaches in these patients.

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Postnatal downregulation of inhibitory neurotransmission to the longitudinal muscle of guinea pig ileum

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Neurogenic contraction and relaxation of ileal longitudinal muscle was studied in tissues from adult and neonatal guinea pigs. Whole mount preparations were maintained in conventional organ baths and drug-induced contractions were measured with isometric force transducers. Myenteric neurons were counted using fluorescence microscopy and an anti-HU antibody. Myenteric neurons containing calbindin (CalB) or nitric oxide (NO) synthase (NOS) and myenteric nerve fibers containing substance P (SP) were also labeled with antibodies and quantified using fluorescence microscopy. In tissues from adult (300–500 g) and neonatal (90–110 g) animals, nicotine (0.3–30 μM) contracted longitudinal muscle in a concentration-dependent manner and TTX (0.3 μM) sensitive manner. In the presence of scopolamine (1 μM), nicotine (3–30 μM) produced smaller concentration-dependent contraction in tissues from adult animals, which was abolished by CP 96,345–01 (0.3 μM), a selective NK1 receptor antagonist. In tissues from neonatal animals, nicotine (0.3–30 μM) did not cause contractions in the presence of scopolamine. When the production of NO was inhibited with Nω-nitro-L-arginine (100 μM), nicotine in the presence of scopolamine (1 μM) caused contractions in neonatal tissues. The density of SP-nerve fibers was not different between adult and neonatal tissues. The ratio of NOS/CalB neurons in the neonatal plexus (1.56 ± 0.08; n = 6) was higher than the in adult (1.22 ± 0.05; n = 6; $P < 0.05$) tissues. The density of myenteric neurons in neonatal (176 cells/mm^2) was higher than in adult (84 cells/mm^2) tissues. The density of myenteric ganglia in neonatal (5 ganglia/mm^2) was higher than in adult tissues (2 ganglia/mm^2) animals. These data indicate that neuronal inhibition of ileal longitudinal muscle is more prominent in neonatal than in adult animals. The more prominent inhibition is due to a higher number of NOS neurons and to a higher neuronal density in neonatal animals.

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Validation and characterization of a model of entero-pancreatic denervation in pigs

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Non insulino dependant diabetes mellitus (NIDDM) is a pathology of increasing prevalence characterized by an impaired glycemic control due to an impaired peripherical tissues sensitivity to insulin as well as an inadequate insulin secretion. Pre absorptive phase of postprandial insulin secretion disappeared in NIDDM. This phase may be controlled by entero-pancreatic (EP) neurons, which directly link the duodenum to the pancreas. To test this hypothesis, we set a model of surgical EP denervation in pigs. The aims of our study were to (1) validate this model and to (2) characterize the EP denervation induced modifications on the pancreatic intrinsic nervous system (INS) and on the structure of the endocrine pancreas. To validate the model, serotonin (5-HT), an EP neurons marker, was looked for by immunohistology in pancreas of intacts (n=13) and EP denerved (n=13) pigs. The characterization of the INS in intact and EP denerved pancreas was also achieved by immunohistology using antibodies directed towards neurotransmitters known to be present in EP neurons and pancreatic INS. Endocrine pancreas structure (% of endocrine tissue, number and size of the islets) was studied by histological colorations. The absence of 5-HT immunoreactive fibers in EP denervated pancreas validated our model of surgical denervation. No difference was found in the endocrine pancreas structure between intact and EP denervated animals. On the contrary, INS of EP denervated pancreas showed a plasticity which was characterized by a lower number of nervous fibers, especially the intrinsic VIPergic ones (containing Vasoactive Intestinal Polypeptide). In conclusion, we validated the surgical entero-pancreatic denervation. This unique validated model will serve as a tool to study the role of EP neurons on the pre absorptive phase of insulin secretion.

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NPY$^{-/-}$ mice resist DSS-induced colitis by reducing oxidative stress

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Recently, neural immune regulation has been identified to play a major role in the development of inflammation in inflammatory bowel disease (IBD). Neuropeptide Y (NPY) is a neuropeptide that plays an important role regulating gastrointestinal motility and feeding behavior. We assessed the role of NPY in modulating the inflammatory response in a murine model of colitis.

Methods: Dextran sodium sulphate (3%DSS in drinking water) was administered to WT and NPY$^{-/-}$ mice for 7 days to induce colitis followed by vehicle for an additional 14 days to evaluate recovery phase. Mice were sacrificed on day 7 and 21 post administration of DSS. Colitis was assessed by clinical index (0–12 measured by weight loss, blood in stool and stool frequency) as well as histology using standard morphological criteria (histological score 0–11). Neutrophil infiltration was assessed by myeloperoxidase activity (MPO). Oxidative stress was assessed by nitrite levels and catalase activity. The functional consequences of inflammation on enteric neurons were assessed by isometric intestinal (circular and longitudinal) muscle recording in conjunction with electrical field stimulation (EFS). **Results:** NPY levels were upregulated in the myenteric neurons of WT-DSS mice. NPY$^{-/-}$-DSS mice had a significantly lower clinical score for colitis compared to WT-DSS mice (NPY$^{-/-}$-DSS: 4 ± 0.8, WT-DSS: 9 ± 0.4, $P<0.01$, n = 6). Histological samples from WT-DSS mice had more severe disruption of colonic mucosal architecture, inflammatory infiltrate and ulcerations compared to NPY$^{-/-}$-DSS (WT-DSS: 6.58 ± 0.30, NPY$^{-/-}$-DSS: 1.75 ± 0.3819, $P<0.05$, n=6). The WT-DSS mice had significantly higher MPO activity compared to NPY$^{-/-}$-DSS ($P<0.01$). Markers of oxidative stress, including nitrite levels ($P<0.05$, n=3) and catalase activity were higher in the WT-DSS mice compared to the NPY$^{-/-}$ DSS mice ($P<0.05$, n = 3). On day 5 there was a significant decrease in the EFS-induced relaxation in WT-DSS mice (3.1 ±

0.2) compared to WT-water (38.62 ± 0.5%) which persisted up to day 21. In contrast, $NPY^{-/-}$ mice did not show any impairment in relaxation suggesting protection from inflammatory damage in these mice.**Conclusions:** We demonstrate, for the first time, that NPY levels are upregulated during colitis. Lack of NPY protects against inflammatory response as well as impaired colonic relaxation. Together, our data suggest that NPY is a critical modulator of intestinal inflammation and may regulate inflammation through increased oxidative stress. NPY and its downstream signal transduction targets may be the potential therapeutic targets in modulating the inflammatory response in IBD.

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Enteric glial protein s100β is upregulated and modulates nitric oxide inflammation in ulcerative colitis

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Introduction: As part of the enteric nervous system, enteric glia participates to gastrointestinal homeostasis. In the human central nervous system increased expression of astroglial derived S100(protein has been associated with nitric oxide (NO) dependent inflammation. It is not known whether enteric glial-S100(protein is involved in the bowel inflammation. **Aim:** To evaluate the expression of S100β protein and its association with nitric oxide production in patients with ulcerative colitis (UC). **Methods:** Rectal biopsies from 30 patients with UC and 40 controls were studied for S100(and inducible NO synthase (iNOS) expression, and nitrite production. To test whether S100(was able to induce NO production, control biopsies were stimulated with exogenous purified S100beta (0.005–5 μM) in the presence of the receptor for advanced glycosilation (RAGE) blocking antibody and iNOS expression and nitrite levels were measured. Similarly, lipid peroxidation and p38-MAPkinase activation were evaluated in the presence of specific inhibitor of p38-MAPkinase and NF-kappa B transcription factor, SB203580 and TLCK, respectively. **Results:** In patients with UC the S100(and the iNOS expression and nitrites were higher than in controls by 399±7.1%, 2537±20% and 247±7.3%, respectively. In cultured control rectal biopsies, S100β induced a 40 fold increase of iNOS expression and a concentration-dependent increase of the nitrite production (65.5± 2.5, 213.8± 4.8, 397.4± 9.0, 749±12.6%); these effects were completely blocked by the specific anti RAGE antibody. The effect of S100β (5 μM) on lipid peroxidation was significantly inhibited by increasing concentration of both SB203580 (0.03; 0.3; 3 μM) [29.7±4.1, 49.3±3.5, 72.6±5.8%] and TLCK (0.01; 0.1; 1 μM) [49.3±4·8, 66.0±5.1, 80±4.4%]. **Conclusions:** We showed that S100(is increased in the rectum of patients with UC. This protein acts via RAGE and directly modulates rectal inflammation through induction of iNOS protein expression and nitrite production. Our data highlight the involvement of glial cell in inflammatory bowel disease.

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Beta-3 adrenoceptor agonism inhibits excitability in human enteric neurons

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Beta-3 adrenoceptors have been suggested to be involved in regulation of motility and visceral analgesia. In this study we investigated the effect of beta-3 adrenoceptor modulation on human enteric neurons. Neuroimaging with the voltage sensitive dyes was used to record fast membrane potential changes in neurons of the human submucous plexus, which was freshly dissected from cancer free regions obtained from patients undergoing colorectal cancer operations. Microejection (400ms duration) of 100μM nicotine evoked fast onset discharge of action potentials at a frequency of 12.2±1.4Hz (n=28). Perfusion of the selective beta-3 adrenoceptor agonist GW427353 at 0.1, 0.3 and 0.5μM significantly reduced nicotine-evoked spike discharge to 7.5±0.8Hz, 4.6±0.6Hz and 2.1±0.3Hz, respectively. The inhibitory effect was reversed after 40min wash out. To test receptor specificity of the response we first perfused the beta-3 adrenoceptor antagonist SR59230A (1μM for 20min) which caused a potentiation of the nicotine-evoked spike discharges from 6.6±0.5Hz to 18.9±1.8Hz (n=77). This was followed by a 20min perfusion of 1μM SR59230A together with 0.5μM GW427353; SR59230A blocked the inhibitory action of GW427353 as the nicotine evoked spike discharge remained high at 22.1±2.5Hz. After 1hr wash out of all substances the nicotine response was still elevated at 19.2±1.2Hz. However, 20–40min perfusion of the beta-3 agonist GW427353 evoked again the inhibitory response and the nicotine evoked spike discharge was significantly reduced to 6.4±0.5Hz. GW427353 also suppressed stimulus-evoked fast excitatory postsynaptic potentials without affecting axonal compound action potentials. Moreover, beta-3 adrenoceptor immunostaining was observed in the neurons studied. About 90% of HuC/D-positive neurons were also beta-3 adrenoceptor-positive. These results suggest that beta-3 adrenoceptor activation causes suppression of neuronal activity in the human enteric nervous system. The potentiating effect of the beta-3 antagonist indicates the presence of a beta-3 adrenoceptor-mediated sympathetic tone that contributes to low excitability of enteric neurons. The beta-3 adrenoceptor-mediated inhibition may decrease the sensitivity of enteric neurons to various stimuli and may be a promising target to reduce neuronal hyperexcitability in the diseased gut.

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The calcium activated K channel of intermediate conductance (IK) modulates excitability of human enteric neurons

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The IK channel is distributed in peripheral tissue including secretory epithelium, blood cells and enteric neurons. A significant decrease of IK expressing enteric neurons has been described in inflamed colon of patients with chronic inflammatory bowel disease. We used neuroimaging techniques with voltage sensitive dyes to study the effects of modulation of IK channels on neuronal excitability in the human submucous plexus freshly dissected from cancer free regions obtained from patients undergoing colorectal cancer operations. Perfusion of the IK channel blocker Tram-34 (5μM) had no prominent effects on stimulus evoked fast excitatory postsynaptic potentials. However, Tram-34 enhanced postsynaptic excitability of human submucous neurons (100 neurons, 7 ganglia, 5 tissues). Microejection of 100μM nicotine for 100ms evoked a spike discharge of 8.5±0.6Hz which was significantly increased in the presence of Tram-34 to 13.8±0.7Hz. After 30min wash out the pre-Tram-34 values were re-established (8.2±0.5Hz). During perfusion of Tram-34 the amplitude and duration of afterhyperpolarisation following spike discharge was significantly reduced. Perfusion of the IK channel opener DCEBIO (5μM) had the opposite effect. The nicotine induced spike discharge rate was significantly reduced from 10.4±0.7Hz to 3.6±0.7Hz and recovered after 45min wash (8.8±1.0Hz). Strong IK channel immunoreactivity was observed in human submucous plexus. Co-labelling with the panneuronal marker anti-PGP9.5 revealed that practically all neurons in the human submucous plexus were IK channel immunoreactive. These results suggest that IK channels are expressed in human submucous neurons where they play a prominent role in modulating membrane excitability and firing properties. IK channels are therefore targets to modulate hyper- or hypoexcitability in the human enteric nervous system.

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Perturbing NMDA receptor – PSD-95 protein interactions prevent selective loss of nitric oxide synthase (NOS) containing neurons in rat gastric myenteric plexus

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Defective accommodation is a serious complication of diabetes. We previously showed that chronic hyperglycemia leads to increased release of nitric oxide, which activates release of glutamate, and this leads to selective damage of NOS containing gastric myenteric neurons. We also showed that NMDA antagonists prevented loss of NOS containing neurons. We hypothesize that activation of glutamate-NMDA-PSD-95-NOS signal pathway leads to nitric oxide mediated damage of NOS containing neurons in the rat stomach myenteric plexus, and that dissociating NMDA receptor from downstream neurotoxic signal may prevent this deleterious effect. To test this hypothesis, we demonstrated via immunocytochemistry (ICH) studies of whole mount preparations of gastric corpus that glutamate containing neurons were abundant and frequently co-localized with NOS or CHAT (acetylcholine) containing neurons. Glia cells containing glutamate synthetase were present in close proximity with enteric neurons. Whole mount gastric corpus ICH of streptozotocin induced diabetic and age matched control rats demonstrated that diabetic animals had a 37.5% reduction of NOS containing neurons but no reduction of CHAT containing neurons. Glutamine containing glia cells also appeared intact in diabetic animals. To demonstrate that NOS containing gastric neurons are selectively damaged by the Glut-NMDA-PSD-95-NOS signal pathway, we constructed Tat-NR2B9c, a peptide that readily transduces cellular cytoplasm and perturbs the NMDA receptor-PSD-95 protein interactions, thereby selectively decreasing glutamate mediated downstream neurotoxic signaling without affecting other glutamate mediated neuronal activities. Exposure of whole mount preparations of gastric corpus to NMDA 1×10^{-5} M for 2 hours resulted in 34% loss of NOS but not CHAT containing neurons. This loss was prevented by Tat-NR2B9c peptide transduction, but not by exposure to Tat 38–48, a peptide that does not transduce cell membrane. In conclusion, we demonstrated an intact gastric myenteric glutamate system in the rat stomach similar to that found in CNS. We also showed that diabetic rats have selective loss of NOS containing neurons in the stomach. Finally, Tat-NR2B9c, a peptide that selectively interrupts the Glut-NMDA-PSD-95-NOS signal pathway without blocking other NMDA receptor functions, prevents loss of glutamate induced NOS containing neurons. This approach to prevent the loss of gastric myenteric NOS neurons may have important therapeutic implications for gastric motility complications of diabetes.

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Kv1 voltage-gated potassium channels in the gastrointestinal tract: distribution and potential mediators of motility dysfunction

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Voltage-gated K^+ (Kv) channels regulate the activity of many cells. We have previously reported autoantibodies to ion channels in dysmotility disorders (Knowles et al., 2002). Kv1 channel distribution in the central nervous system and cardiovascular system is well characterised, but very little is known of Kv1 channel distribution and function in the gastrointestinal tract. In the present study we have investigated the distribution of six major Kv1 channel subunits in mouse gastrointestinal tract, and human ileum and colon. **Methods:** Indirect immunohistochemistry was performed on formalin-fixed, paraffin embedded sections of murine stomach, small bowel and colon using the avidin-biotin peroxidase complex (ABC) method. Polyclonal anti-Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5 and Kv1.6 were used as primary antibodies. Sections were examined by light microscopy and staining scored by two investigators after reaching consensus and independently by an experienced third investigator. **Results:** Significant variation was seen in the distribution of subunits in different parts of the bowel wall ($p<0.05$). The greatest concentration of Kv1 subunits was found in the intestinal surface epithelial cells, gastric chief cells and enteric ganglia. Kv1.1, 1.2, 1.3, 1.4 and 1.5 immunoreactivities were similar in small bowel surface epithelium whereas Kv1.6 showed lower intensity. Colonic surface enterocytes were intensely stained with anti-Kv1.2, Kv1.3 and Kv1.4 antibodies. In the stomach, Kv1.1, Kv1.2 and Kv1.3 immunoreactivities were prominent in chief cells. Gastric myenteric ganglia also showed strong immunoreactivity for Kv1.3. Submucosal ganglia of the small bowel were strongly immunopositive for all subunits. Myenteric ganglia showed the same levels of immunoreactivity as seen in the surface epithelium. Colonic ganglia were at least moderately positive for all six subunits. Gut smooth muscle, including vascular smooth muscle was moderately or weakly positive for Kv1 subunit expression. **Conclusions:** This is the first comprehensive description of the distribution of voltage-gated K channels throughout the gastrointestinal tract. The high density of Kv1 subunits in surface epithelial cells and enteric ganglia was unexpected. The demonstration of a common antigenic profile provides a potential mechanism whereby damage to enterocytes could produce an immune response with enteric neuron cross reactivity leading to impaired motility, for example in post infective irritable bowel syndrome.

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Mechanisms of synaptic facilitation in the myenteric plexus of the inflamed guinea pig distal colon

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Inflammation has been shown to affect various aspects of enteric phsyiology, including facilitation of fast synaptic communication in the enteric nervous system. To determine the mechanisms responsible for this facilitation, intracellular recordings were obtained from S neurons of control and trinitrobenzene sulfonic acid (TNBS) – treated (6 days) guinea pig distal colons. Responses to exogenously applied neurotransmitters, as well as fast synaptic potentials (fEPSPs) were evaluated. No change in the response to the exogenously applied neurotransmitters acetycholine or ATP was identified, suggesting that synaptic facilitation involves a presynaptic mechanism. Alterations in the pharmacology of individual synapses, or in the proportion of types of synapses in inflamed tissue were not detected, indicating no involvement of neurotransmitter recruitment. This led us to investigate how inflammation may affect the neurotransmitter release mechanism. Rundown properties during 20 stimuli at frequencies of 0.5, 5, 10, and 20 Hz. were evaluated. Unlike the ileum, complete rundown did not occur in the distal colon, and was less extensive in inflamed tissue (control, 44.4 ± 9.0%, n = 10; colitis, 67.9 ± 8.2%; n =11; 20 Hz). Release properties were evaluated using a paired pulse stimulation paradigm at a 50 msec latency. Paired pulse depression was detected in control tissue (ratio of EPSPs of $2^{nd}/1^{st}$: 0.8 ± 0.1, n=21), whereas paired pulse facilitation (ratio = 1.2 ± 0.1, n=24) was detected in colitis, suggesting the probability of transmitter release decreases in colitis. Activation of protein kinase A (PKA) leads to an augmentation of synaptic events in the ENS. Therefore, the specific PKA inhibitor, H89 (10 μM), was applied to the bathing solution to evaluate the relative effects of PKA inhibition on EPSPs in normal and inflamed tissue. No change in the fEPSP amplitude was detected in control tissue after application (Krebs, 21.7 ± 3.2; H89, 20.2 ± 2.8, n = 3), however, after application in inflamed tissue the fEPSP amplitude returned to normal amplitudes (Krebs, 30.6 ± 4.2, H89, 20.6 ± 3.5, n = 6). These findings demonstrate that synaptic facilitation in colitis involves a presynaptic mechanism that does not involve an alteration in the types of transmitter released, but rather a fundamental change in transmitter release properties. Additionally, PKA appears to be activated in nerve terminals of the inflamed colon, and likely contributes to the facili-

tation of myenteric fEPSPs in the inflamed distal colon. Supported by DK62267.

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Mechanisms behind the secretory response to distension in distal rat duodenum *in vivo*

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Aim: Distension of rat proximal small intestine generates a complex secretory response. The aim of the study was to characterize the underlying neural mechanisms. **Methods:** Experiments were performed in chloralose-anaesthetized male rats. A 5–6 cm long segment of distal duodenum was distended with saline, and the resulting change in transmucosal potential difference (PD) was recorded. The segment was distended for 5 minutes at 5, 10, 20, 40, 60 and 80 mmHg with 10 min intervals between distensions. The following substances were used to block different components of the putative neural circuitry: SR140333 (3 μmol/kg): blockade of NK1 receptors; Serosal lidocaine (0.5 mg/10 cm of intestine, applied on the serosa every 10th minute): blockade of myenteric plexus; Hexamethonium (10 mg/kg): blockade of nicotinic receptors; (4Cl-D-Phe^6, Leu^{17})-VIP (2 $\mu g^{\star}kg^{-1\star}min^{-1}$): blockade of VIP receptors and Granisetron (40 μg/kg): blockade of 5-HT3 receptors. **Results:** In control animals (n=26), luminal distension induced a biphasic response consisting of an initial peak (activation component) followed by a sustained response. SR140333 significantly inhibited both the activation component and the sustained component. Serosal lidocaine and hexamethonium reduced the activation component but had no effect on the sustained component. The VIP receptor antagonist did not affect the activation component, but inhibited the sustained component at pressures 5, 10 and 20 mmHg. Hexamethonium + the VIP receptor antagonist given together reduced the activation component and abolished the sustained component. Granisetron enhanced both the activation component and the sustained component. **Conclusions:** The activation component seems to be mediated by NK1 receptors and nicotinic receptors. The effect of serosal lidocaine suggests a major contribution of the myenteric plexus in the activation component. The sustained component was also mediated by NK1 receptors, but was resistant to lidocaine, suggesting involvement of the submucous plexus. VIP receptors seem to play a major role in the submucous system, acting together with cholinergic (nicotinic) transmission from the myenteric plexus to activate the circuit. A prerequisite for this model is that the VIP antagonist used preferentially acts on neural VIP receptors rather than the final secretomotor effector step.

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Hydrogen sulfide is synthesized in the muscle layers of the colon and in prevertebral ganglia and alters the electrical properties of neurons

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Hydrogen sulfide (H_2S) is a newly appreciated modulator of excitable tissues that is involved in vasorelaxation, synaptic plasticity, and neurogenic inflammation. We hypothesized that H_2S is relevant to the physiology of gastrointestinal tissues. While previous studies have demonstrated that gastrointestinal tissue expresses the enzymes that synthesize H_2S, cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS), and tissue homogenates are capable of generating H_2S, these studies have not excluded the contribution of enteric flora to these measures, nor demonstrated H_2S production in intact tissue preparations. RT-PCR and/or immunohistochemistry indicate that CSE is expressed in the muscularis externa of mouse and guinea pig colon. CSE is most highly expressed in neurons of the myenteric plexus as well as smooth muscle cells and ICC. Both CSE and CBS are expressed in the muscle layers of the human colon. Sympathetic prevertebral ganglion neurons from mouse and guinea pig express CSE. Using intestinal muscle preparations never directly exposed to luminal contents, H_2S production from intact tissues was assessed using gas chromatography. Intestinal tissues were capable of generating H_2S at levels nearly three fold higher than control solutions containing L-cysteine, the substrate for CSE and CBS. The effect of the H_2S generating molecule, NaHS, on mouse superior mesenteric ganglion neurons was determined using intracellular electrophysiology. Of twelve cells tested, three did not respond to 1mM NaHS, three cells responded with a prolonged hyperpolarization of 10 ± 3 mV, three cells responded with a depolarization of 3.5 ± 0.8 mV, and three cells exhibited a biphasic response of a short hyperpolarization (2.3 ± 0.6 mV) and prolonged depolarization (2.8 ± 0.6 mV). All responses were delayed in onset with an average latency of approximately 20 s. Collectively, these data indicate that the muscle layers of the colon and cells in prevertebral ganglia are capable of producing H_2S likely through enzymatic reactions involving CSE and CBS. Furthermore, these data indicate that H_2S can affect neuronal excitability as both hyperpolarizing and depolarizing responses are elicited by exogenous NaHS. It is therefore highly likely that H_2S plays a physiological role in gastrointestinal function. Supported by DK17632 and DK17238.

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Peripheral CRF_1 receptor activation mediates cold-restraint stress-induced c-fos expression in enteric neurons in the guinea-pig colon

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Background: Corticotropin releasing factor (CRF) has been implicated in stress-evoked gastrointestinal functional changes through both central and peripheral mechanisms. The present study focused on the peripheral mechanisms by testing a hypothesis that CRF acts in the enteric nervous system (ENS) to alter gut motor and secretory responses during stress. **Methods:** Male adult guinea-pigs were placed under cold-restraint stress (CRS) for 2 h. Controls were allowed to move freely in their cages at room temperature. Fecal pellet output (FPO) was monitored for 2 h. Animals were euthanized immediately at the end of 2h. Segments of proximal (PC) and distal colon (DC) were removed. Whole-mount myenteric and submucosal plexus preparations were used for c-fos staining and double labeling of c-fos with CRF_1 receptor, choline acetyltransferase (ChAT), substance P (SP), calbindin, nitric oxide synthase (NOS) and vasoactive intestinal peptide (VIP). **Results:** Exposure to CRS for 2 h significantly increased the number of c-fos immunoreactive (IR) neurons in the myenteric and submucosal plexuses of the PC and DC (13.0 ± 3.2 vs 2.5 ± 1.2 in PC myenteric; 10.0 ± 1.8 vs 3.7 ± 1.1 in PC submucosa; 14.7 ± 2.6 vs 2.7 ± 1.0 in DC myenteric; 9.7 ± 0.8 vs 4.7 ± 1.2 in DC submucosa). The majority of the c-fos-IR neurons were surrounded by CRF_1 receptor immunoreactivity. Intraperitoneal (i.p.) injection of the selective CRF_1 receptor antagonist NBI 27914 (10mg/kg) 30 min prior to CRS suppressed c-fos expression in enteric neurons, while i.p. CRF_2 receptor antagonist antisauvagine-30 (100 μg/kg) had no effect. CRS induced c-fos expression in ChAT, SP, calbindin, and NOS-IR neurons in the myenteric plexus, and ChAT and VIP-IR neurons in the submucosal plexus. CRS increased FPO/2 h and water content in the feces, which were attenuated by i.p. NBI 27914, but was unaltered by antisauvagine-30. **Conclusions:** Acute CRS induces c-fos expression in colonic enteric neurons through stimulation of the CRF_1 receptors in the ENS. The CRF_1-mediated activation of certain neurochemically defined neural pathways may lead to disturbances in gut motility and secretion during stress. (Supported by a Pharmaceutical Manufactures of American Foundation Postdoctoral Fellowship to S. Liu, NIH RO1DK37238 to J.D. Wood and KO8 DK60468 to Y. Xia)

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GDNF mediated Enteric Neuronal Survival involves GSK3beta and Tau

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Background and Significance: Glial cell line-derived neurotrophic factor (GDNF) is a growth factor required for enteric neuronal cell survival. However, the mechanism of how GDNF promotes enteric neuronal survival remains to be established. We have previously demonstrated that GDNF promotes their survival through activation of the PI-3-kinase pathway. Here we examined the role of one of the downstream targets of PI-3-kinase, GSK3β, a serine threonine kinase whose activity is inhibited by Akt, in mediating the effects of GDNF. **Methods:** Enteric neurons were isolated from digested rat embryonic intestines by immunoselection with a NGF receptor antibody (p75 NTR). They were exposed to GDNF either in the presence or absence of the PI-3-kinase inhibitor LY294002 (LY, 50μM). The amount of phospho-GSK3β and phospho-tau was assessed by Western blot analysis using specific antibodies. Apoptosis was assessed using Western blot analysis or cleaved caspase-3 immunocytochemistry. **Results:** GDNF enhanced the phosphorylation of GSK3β and this increase was lost in the presence of LY ($P<0.05$). 14-3-3 is a protein known to interact with the down stream targets of Akt. We examined if GDNF could promote the association of 14-3-3 with GSK3β in enteric neurons. Enteric neurons were exposed to vehicle, GDNF (100ng/ml) or GDNF + LY for 30 minutes and total protein extracted. GSK3β was immunoprecipitated from these extracts and an immunoblot for 14-3-3 performed. GDNF promoted the interaction of 14-3-3 with GSK3β and this was inhibited by the PI-3-kinase inhibitor, supporting the importance of the Akt-mediated GSK3β/14-3-3 complex formation in the survival pathway. Overexpression of a Wt GSK3β construct or a GSK3β/S9A contruct mutated at one the Akt phosphorylation sites resulted in enhanced enteric neuronal cell death as assessed by immunocytochemistry ($P<0.001$ compared to control vector) and Western blot analysis ($P<0.01$, compared to control vector) for cleaved caspase-3. Transfection of a constitutively active S9A-GSK-3β mutant prevented the survival effects of GDNF, whereas a dominant negative GSK-3β construct prevented GDNF withdrawal-induced cell death. Increased GSK-3β activity was associated with an increase in tau phosphorylation. **Conclusions:** Thus, GDNF promotes enteric neuronal survival by modulating GSK-3β and its downstream target tau. Inhibitors of GSK-3β activity may have therapeutic potential in improving enteric neuronal survival.

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Neuroprotective effects of enteric glial cells in a model of dopamine induced-neurotoxicity

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Introduction: Neurodegeneration of the enteric nervous system (ENS) has been reported during various intestinal inflammatory conditions and neurodegenerative disorders of the central nervous system. In addition, recent studies suggest that enteric glial cells (EGC), one of the major components of the ENS, may have a neuroprotective effect. Therefore, the goal of the study was to determine the putative neuroprotective role of EGC during dopamine (DA)-induced neurotoxicity. **Aims & methods:** Primary cultures of enteric neuronal cells from rat embryos and cultures of the human neuroblastoma cell line (SH-SY5Y) were used to characterize DA-induced neurotoxicity. The neuroprotective effect of EGC was studied by co-culturing primary cultures or SH-SY5Y with rat EGC. Neuronal cell death was evaluated by measuring: 1) Neuronal Specific Enolase (NSE) release, 2) tetrazolium salts (MTT) conversion and membrane permeability (propidium iodide, 7-Amino-Actinomycin D) by FACS. The $[Ca^{2+}]_i$ changes were assessed by Fluo-4 imaging and mitochondria were visualized with Mitotracker green. **Results:** In primary cultures of ENS, DA treatment (1.2 mM for 24h) induced major alterations in the neuronal network associated with a significant increased of NSE release in the culture medium from 0.8±0.1 to 4.1±0.3 ng/ml (n=7, $p \leq 0.001$). In SH-SY5Y, DA induced a time and dose dependent neuronal cell death characterized by an 80% decrease of MTT conversion, and a 7-fold increase of membrane permeability. The K^+-induced $[Ca^{2+}]_i$ rise observed in control SH-SY5Y cells was abolished upon treatment with DA (8h incubation) and their mitochondrial network was disrupted. Co-cultures of ENS with EGC significantly reduced DA-induced NSE release (n=6, $p \leq 0.05$). Direct co-cultures of EGC and SH-SY5Y prevented DA-induced alterations of membrane permeability and partially restored the K^+-induced $[Ca^{2+}]_i$ transients. **Conclusion:** This study identified a neuroprotective role for EGC during DA-induced neurotoxicity. The factors and mechanisms involved remain to be identified.

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Developmental regulation of the neurochemical phenotype of the ENS: implication of l-type ca2+ channels

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Introduction: The developmental and activity dependent regulation of the phenotype of the ENS remains largely unknown. Therefore, we aimed to characterize in vitro the phenotypical changes of enteric neurons and their modulation by KCl in an embryonic model of rat and mouse primary culture of ENS. **Aims & Methods:** Following tissue trypsination of small intestine of rat or mouse embryos (E15), the cell suspension was seeded in 12-well plates. Following culture in absence or presence of KCl (40mM), cells were processed for immunohistochemistry or quantitative PCR. Immunohistochemical studies were performed using antibodies against PGP9.5, VIP, nNOS, TH. Pharmacological studies were performed with L-type Ca2+ channel blocker (Nifedipine 1μM), an agonist of L-type Ca2+ channel (Bay-K8644 1μM). The effects of norepinephine (1μM) and clonidine (1μM) upon the phenotype were also evaluated. **Results:** The number of PGP9.5 immunoreactive (-IR) neurons/ganglia increased over time from 3±1 at day 1 (D1) to 35±6 at D13 (n=7) in rats. Over those 13 days, there was a significant decrease in the proportion of VIP-IR and nNOS-IR neurons. At D1, 94±2% and 100±0% of PGP9.5-IR neurons were VIP- and NOS-IR, respectively. At D13, these proportions dropped to 30±8% and 33±7%, respectively. Tyrosine Hydroxylase(TH)-IR neurons were present at D1 (28±1%), but vanished (0±0%) as early as D7. After 13 days of culture with KCl, major changes in the phenotype were observed. There was a significant increase in the proportion of TH- and VIP-IR as compared to control (52±4% TH-IR neurons vs 1±0%, respectively, $p<0.001$, n=11 and 41±2% VIP-IR neurons vs 29±3%, p=0.007, n=4). These changes in the phenotype were associated with an increase in TH and VIP mRNA expression (51 fold, p=0.032 and 40 fold, p=0.031, n=5, respectively). The proportion of nNOS-IR neurons was unchanged by KCl. Addition of KCl at D10 for 72h increased the proportion of TH-IR neurons as compared to control (22±1% vs 1±0%, $p<0.001$, n=8). Similar results were obtained in the mice primary cultures of ENS. The increase in TH expression was significantly blocked by nifedipine (μM), while Bay-K8644 significantly increased the proportion of TH-IR neurons as compared to control (8±1% vs 1±0, p=0.002 vs control, n=6). Finally, addition of norepinephrine and clonidine significantly reversed the changes in TH phenotype induced by KCl. **Conclusion:** sThis study suggests that KCl modulates the phenotype of a specific neuronal population in part via L-type Ca2+ channel. The mechanisms and factors involved in this regulation remain to be identified.

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Vagotomy affects intrinsic neural responses in the guinea pig stomach

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The vagus nerve carries central control to the gastric enteric nervous system with nitric oxide mediating fundic accommodation and acetylcholine mediating antral contractility. Truncal vagotomy leads to impaired proximal gastric accommodation with enhanced liquid emptying and impaired antral contractility with delayed solid emptying. The effects of vagotomy on intrinsic neural responses in the antrum and fundus are not well described.

Aim: To investigate the effects of vagotomy on intrinsic neural responses of fundic and antral smooth muscle. **Methods:** Three groups of adult male guinea pigs were studied: normal controls, truncal vagotomy (1 week post surgery), and surgical controls after sham vagotomy. Fundic and antral circular muscle strips were prepared for in vitro studies. Electric field stimulation (EFS; 16 Hz, 60 sec) was used to stimulate enteric nerves. The effects of the nitric oxide synthase (NOS) inhibitor (L-NNA 100 μM) and atropine 1 μM were assessed. NOS-containing neurons were identified immunohistochemically with full thickness strips. **Results:** NOS-containing cells were identified in the myenteric plexus of the fundus and antrum in similar numbers for normal controls, sham surgical controls, and vagotomized animals. In the fundus, EFS produced relaxation in normal controls (-0.22±0.03 kg/cm^2) and sham surgical controls (–0.29±0.05 kg/cm^2), but contractions (+0.45±0.21 kg/cm^2; p<0.01) in the vagotomized animals. L-NNA converted EFS-induced fundic relaxations to contractions in normal controls (1.97±0.34 kg/cm^2) and sham surgical controls (1.24±0.19 kg/cm^2). L-NNA augmented EFS-induced fundic contractions in vagotomized animals (1.52±0.24 kg/cm^2). In the antrum, EFS produced contractions in normal controls (0.42±0.14 kg/cm^2), sham surgical controls (0.30±0.09 kg/cm^2), and vagotomized animals (0.31±0.10 kg/cm^2). L-NNA increased EFS-induced antral contractions in all 3 groups although the increase was significantly less in vagotomized animals. Atropine inhibited fundic and antral contractions in the presence of L-NNA. **Conclusions:** Truncal vagotomy leads to altered intrinsic neural responses in fundic and antral smooth muscle. EFS-induced fundic relaxation due to activation of intrinsic NOS nerves is changed to contraction after truncal vagotomy. The increase in EFS-induced contractions was less from L-NNA in the antrum from vagotomized preparations than in normal and sham controls. Thus, the impaired ability of the fundus to relax and the antrum to contract in patients after surgical vagotomy may be related to alterations in the enteric nervous system and its effects on gastric motility.

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Aging of the intrinsic and extrinsic innervation of the gastrointestinal tract of the F344 rat

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Gastrointestinal disorders affecting the elderly appear symptomatic of declines in autonomic function. The age-related neuronal losses in the enteric nervous system (ENS) that have been described repeatedly are consistent with this premise. Little, however, is known about either the timetable or the specificity of this enteric cell death, and even less is known about the effects of age on the sympathetic efferent and visceral afferent projections to the ganglia that undergo cell loss. The goal of the present study, therefore, was to describe age-associated changes in both the ENS and its innervation from extrinsic sources. Whole mounts of the stomach, small intestine and large intestine from ad libitum fed virgin male Fischer 344 rats sampled at various ages (e.g., 8, 16, and 24 months of age) were surveyed. A battery of stains, antibodies, and tracers was used to identify and characterize neuronal profiles and axon terminals. The onset of neuronal loss in the myenteric plexus (MP) starts in adulthood, progresses in a linear manner across the lifespan of the rat, and is specific to cholinergic neurons. A concurrent loss of enteric glia that support myenteric neurons is observed in aged rats. Though the number of nitrergic neurons in the MP does not decrease with age, axonopathies (e.g., markedly swollen axons) are evidenced by some nitrergic axons terminating within the plexus and some projecting to smooth muscle. The submucosal plexus undergoes neuronal losses with age that parallel those found in the MP, but it remains to be determined whether submucosal cell death is specific to a particular chemical phenotype, as is the case in the MP. Axonal swellings, dilated varicosities, decreases in tyrosine hydroxylase-immunoreactivity, and axonal losses occur in the sympathetic innervation of the ENS of aged rats. The visceral afferent innervation of the ENS exhibits similar compromises, however the axonopathies in these afferents appear both to have a much later onset in the lifespan of the rat (i.e., 16 months of age) and to be less severe than the neuropathies observed in aging sympathetic projections. The dramatic and consistent pattern of losses characterizing ENS aging is consistent with the supposition that deterioration of the nervous system of the gastrointestinal tract is one possible mechanism for the age-related decline in function evidenced in the elderly.

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Peripheral glia regulate epithelial permeability at mucosal surfaces via release of *s*-nitrosoglutathione

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Barrier functions across epithelia and endothelia are essential for homeostatic tissue regulation. Astroglia interact with cerebral endothelia to maintain the blood-brain barrier. Whether similar interactions between peripheral glia and epithelia regulate mucosal barrier function is not known. Here, we demonstrate that ablation of peripheral enteric glial cells in transgenic mice causes intestinal mucosal barrier dysfunction resulting in inflammation. Glial-derived s-nitrosoglutathione (GSNO) was identified as a potent inducer of mucosal barrier function in vitro and in vivo, and attenuated tissue inflammation following ablation of enteric glia in transgenic mice. GSNO upregulated epithelial tight-junction associated proteins ZO-1 and occludin, and significantly restored mucosal barrier function in colonic biopsies from patients with Crohn's disease, a well described inflammatory permeability disorder associated with enteric glial-cell disruption. Thus, peripheral glia share the ability of astrocytes to regulate tight-junction integrity, and cellular interactions comparable to those maintaining blood-brain barrier function regulate epithelial permeability at mucosal surfaces.

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Substance P and VIP are low in right colon in pediatric Slow Transit Constipation (STC)

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Background: Studies using visual assessment show reduced substance P (SP)- containing nerves in colon in STC children. Numbers of enteric neurons decrease with age, suggesting the age of control samples is crucial. We used quantitative analysis to measure densities of nerve fibres containing SP, vasoactive intestinal peptide (VIP) or nitric oxide synthase (NOS) in colonic circular muscle from (1) children with STC confirmed by scintigraphy (nuclear transit study), (2) constipated children without colonic dysmotility and (3) adults with colorectal cancer. **Methods:** Children with chronic treatment-resistant constipation had scintigraphy. Laparoscopic seromuscular biopsies (circular and longitudinal muscle) were from right (RT) and left transverse (LT)

and sigmoid colon from 51 children with proven **STC** [35M, mean 8.2 (2.0–14.8) yrs] and 20 constipated children without colonic dysmotility [**Non-STC**, 16M, mean 5.6 (0.1–16.8) yrs]. Control tissue was from the normal margin of colon from **adult** colorectal cancer patients (n = 33) and 3 children with Familial Adenomatous Polyposis (**FAP**). Fluorescence immunohistochemistry was performed, using antibodies to NOS, VIP and SP. Density of nerve fibers were quantified in circular muscle in confocal images.**Results: 1. Children vs adults:** All pediatric groups had higher densities SP or NOS nerve fibres than adults ($p<0.05$), Non-STC children had higher VIP than adults ($p<0.05$). STC children had lower VIP than Non-STC children ($p<0.05$) and similar to adults. **2. Regional variation:** FAP children had different density of neurotransmitters along the colon, with highest density in sigmoid colon. **3. Constipation children:** 12/50 (24%) STC children had low SP-immunoreactivity on visual assessment. On quantitation, STC children had reduced VIP and SP in RT compared to Non-STC children ($p<0.05$). When all regions were pooled, reductions in SP in STC children were not significant. **Conclusions:** 1. Comparing density of nerve fibres containing transmitters in pediatric patients with adult colorectal cancer patients is not valid due to age-related reductions in enteric nerve fibers. 2. There are regional differences in density of each transmitter and assessment of regional variation is important in pathology. 3. Pediatric STC is associated with low SP or low VIP in the right colon. 4. Visual identification of decreased SP-immunoreactivity in STC children was confirmed quantitatively.

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Esophageal manometric abnormalities in Parkinson's disease

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The esophagus is frequently involved in neurological degenerative disease such as Parkinson's disease, which affects 0.25% of the general population. Dysphagia, for solid and liquids foods is present, especially related to oro-pharyngeal dysfunction, but there are some data on the involvement of the esophageal body, too. We performed a manometric, videoesophageal and radionuclide study in 35 patients with various stages of Parkinson's disease–8 stage I, 9 stage II, 13 stage III, 4 stage IV and 1 stage V–using the classification Hoehn and Yahr. Dysphagia was present especially in stage III and IV. Manometric abnormalities were found in 71% and were represented by simultaneous contractions, repetitive contractions, high-amplitude contractions, reduced LES pressure, even when they have a clinical expression or not. The reported frequency of swallowing abnormalities in 50% of these patients is not confirmed by the present videoesophagographic study. Also radionuclide evaluation of motor esophageal disorders in Parkinson's disease is useful, especially in stages II and III of the disease.

In conclusion, we have shown that motor abnormalities and esophageal symptoms are present in Parkinson's disease, however non specific pattern was found. A special discussion should be made regarding the medication, our patients being studied during an off period by withholding their drugs on the morning of manometric tests. Our purpose in a further study is to make a comparison between motor abnormalities in on-off drug periods.

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Expression of TRPV1 and $P2X_3$ in vagal and spinal pathways following acid-induced esophagitis in rats

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Background: Esophageal hypersensitivity is one of the common findings in gastro-esophageal reflux disease (GERD). Transient receptor potential vanilloid 1 (TRPV1) and $P2X_3$ purine receptors contribute significantly in hypersensitivity. The aim of this study was to evaluate the altered expression and co-expression of two receptor molecules in the spinal and vagal afferents in esophagitis. **Method:** Four groups of rats (n=3 in each group) were used in this study. In group 1, 2 and 3, fundus was ligated by suturing the limiting ridge between the fundus and corpus and placing a nalaton ring 2cm below the pyloric sphincter to restrict gastric emptying. Group 4 rats (sham control) received a midline abdominal incision, but no ligation. Group 1 was sacrificed 48 hrs after surgery and groups 2, 3 and 4 were sacrificed 7 days after surgery. Bilateral nodose ganglia (NG), spinal cord (T2–T4) and respective dorsal root ganglia (DRG) were collected for examining TRPV1, $P2X_3$ and IB4 immunoreactivity (ir). The Group 3 rats received proton pump inhibitors (omeprazole 20mg/kg/day, p.o.) for 7 days starting on the day of surgery. In addition, esophagi were collected from non-perfused rats (n= 3 in each group) for myeloperoxidase (MPO) assay as a marker of neutrophil infiltration in inflammation. **Results:** Fundus ligation produced severe inflammation of the distal esophagus. In DRG, $P2X_3$-ir cells were significantly high in 48 hrs and 7days ligated rats compared to sham operated and PPI-treated rats ($p<0.05$). Interestingly, the number of IB4-positive cells in DRG also increased in ligated rats (48hrs and 7 days vs in sham, $p<0.01$). In NG, TRPV1-ir and $P2X_3$-ir cells in 7 days ligated rats were significantly higher in comparison to sham operated controls ($p<0.01$). Like in DRG, IB4-positive cells in NG were significantly higher in ligated rats (48hrs and 7 days) compared to sham and PPI-treated groups. **Conclusions:** This study provides a strong evidence for the involvement of TRPV1 and $P2X_3$ channels in reflux-induced changes in vagal and spinal afferents. Results indicate that following inflammation there is a phenotypic change in number of IB4-positive cells in DRG and NG. These changes can be attenuated by PPI. Therefore, it can be speculated that acid can contribute to sensitization of primary sensory neurons by upregulating ionotropic channels.

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Pharmacological separation of the gastro-esophageal segment into three distinct sphincteric components

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The gastroesophageal sphincter contains additive contributions from smooth muscle (SM) intrinsic to the wall and from an extrinsic skeletal crural diaphragm (CD). The attachments between the costal and CD and esophageal wall change the relative alignments of intrinsic-extrinsic sphincters with respiration. We quantify the separate contributions to the high-pressure zone by pharmaceutically suppressing one or the other of the skeletal/SM components during superior/inferior diaphragmatic excursion. **Methods.** Pull-throughs of concurrent manometry and high-frequency ultrasound (US) were carried out in 15 normal subjects during breath-hold at full inspiration (FI) and full expiration (FE). Pull-throughs were repeated after intravenous atropine to suppressing ~50% of basal tone and isolating skeletal muscle tone. The suppressed SM pressure was reconstructed by subtracting post-atropine from full pressure profiles, referenced to the crus (US).(II) 7 patients undergoing general anesthesia for nonesophageal pathology were given cisatrcurium to paralyze the crus. After lung inflation to displace the diaphragm, pull-throughs were performed and SM pressures measured. **Results.** The CD moved 2 cm between FI and FE. The high-pressure zone was broader in FE. The atropine-suppressed pressure peaks were correlated with the crus muscle position ($R^2 = 0.83$). Subtracted pressure profiles were double peaked and matched closely double-peaked profiles from the post cisatracurium SM data. The proximal SM peak moved rigidly with the CD while the two intrinsic pressure peaks separated 1.1 cm with diaphragmatic displacement. The correspondence between the two protocols and the anatomical correlation of the atropine-ablated sphincter to the crus muscle indicates that the atropine-ablated and the subtracted pressures reflected the separate contributions from skeletal/SM. **Conclusions.** The SM sphincter has upper and lower LES components associated with the two pressure peaks. The ''upper LES'' is rigidly attached to the diaphragm by phreno-esophageal ligaments and overlaps with the CD. Since the muscle of the upper LES is not anatomically distinct, its tone is presumably physiological in nature. The ''lower LES,'' associated with the lower pressure peak, appears to correlate with the sling-clasp muscle groups at the thickened esophago-cardiac junction. We conclude that the gastro-esophageal sphincter has three distinct

components, two intrinsic SM components and one extrinsic skeletal muscle component, each of which contribute in different ways to the reflux barrier as the physiological and mechanical state of the gastro-esophageal environment varies. NIH grant R01DK59500.

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Three-dimensional anatomy of the human gastro-esophageal segment

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The sphincter within the gastro-esophageal segment (GES) functions via a complex arrangement of components. The search for an anatomical intrinsic sphincter has lead some researchers to describe an axial thickening at the esophago-cardiac junction in cadavers. Previous quantifications of *in vivo* anatomy of the GES have considered only average esophageal wall thickness along the lumen (x), averaged from 3 or 4 azimuthal locations (θ), from ultrasound. Our aim was to quantify the full **t**hree-dimensional (x-θ) anatomy of the GES *in vivo*.
Methods: High-frequency endoluminal ultrasound (US) was co-localized with manometric pressure. The catheter assembly was drawn from the stomach through the GES at 5 mm/s with breath held at full inspiration (FI) and full expiration (FE). Using a knowledge-based image segmentation system, images were filtered to remove noise and the coordinates (R,θ) of the inner/outer muscle wall boundaries were accurately quantified. The muscle and mucosae thicknesses were quantified at 100 θ angles for each of ~20 images per pull-through, every 4 mm, spanning roughly 7 cm segments. Data were referenced to the inferior margin of the crural diaphragm. The time-intensive nature of image segmentation limited quantification to 9 healthy volunteers (24–45 yrs). **Results:** On average, the inferior margin of the crural diaphragm was 3 mm (FE) to 5 mm (FI) below the mid high-pressure zone. The azimuthal variation in muscle thickness was extremely high in all subjects. At each axial location the maximum/minimum muscle thickness varied from 150%-535%. The average of the largest ratios of the largest max/min muscle thickness over the 9 pull-throughs was 347%, and the average of the smallest ratios was 175%. Minimum muscle thickness varied from 0.75–0.90 mm (avg 0.84 mm), while maximum muscle thickness varied from 3.2 to 4.4 mm (avg 4.0 mm). Ensemble averages of the θ-averaged muscle thickness decreased from the stomach to thoracic esophagus (2.4 mm at -1 cm to 1.6 mm at +3 cm), as did mucosal thickness (4.0 mm at -1 cm to 2.6 mm at +3 cm). There are no significant differences in thicknesses between FI and FE. Azimuthal asymmetry was greatest in regions with direct contact with the crus muscle. **Conclusions:** The esophageal wall in the resting GES is highly three dimensional in thickness and cross-sectional geometry, due to the external impingement of the crus muscles. The extremely large azimuthal variation in muscle layer thickness makes it necessary to quantify muscle thickness vs. θ to obtain accurate estimates of average muscle thickness. Averages obtained from only a few θ locations have high levels of uncertainty. Consistent with cadavers, *in vivo* muscle and mucosal thicknesses increase continuously through the GES to the esophago-cardiac junction. Supported by NIH R01 DK56033 (JGB), R01-DK59500 (LEM, QD); Bard Endoscopic Tech (RU).

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Distinguishing GERD: Slight distension of the gastro-esophageal segment greatly enhances the probability for reflux, but compliance does not

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Gastro-esophageal reflux generally occurs during transient LES relaxation (tLESR); yet high tLESR frequency does not imply GERD. Mechanical relationships within the gastro-esophageal segment (GES) must therefore play a role. Compliance has been suggested as a distinguishing characteristic, and a recent study found that the GES radius in GERD patients without hiatal hernia was ~1 mm higher above normal. We apply physics-based mathematical modeling to evaluate sensitivities to compliance and distension at the initiation of opening.
Methods: A mechanically accurate mathematical model of the GES during opening was developed from Newton's laws applied to the two muscle layers and to retrograde flow of gastric liquid. The high pressure zone (HPZ) contribution was specified and the muscle passive stress-strain properties were parameterized from *in vivo* ultrasound-manometry data. Intrathoracic pressure was atmospheric, and baseline abdominal pressure taken as 5 mmHg above thoracic. Transmural pressure differences were initially zero. Gastric pressure was suddenly raised by a specified amount, DP (1–4 mmHg) and the model predicted opening and flow. ''Reflux'' was defined as ''at least 2 ml of gastric liquid entering the esophagus within 1 s.'' Predictions of opening and reflux were made as a function of an initial ''film thickness,'' T, of liquid coating the GES (distension), and material stiffness of the GES (compliance). The ''reflux-no-reflux boundary'' was defined—for specified GES distension, GES compliance, and gastric pressure rise DP—by adding and increasing a tonic component until reflux was suppressed. **Results:** ''Opening'' does not necessarily imply ''reflux.'' Rate of opening determines reflux and depends on DP relative to tone. The reflux-no-reflux boundary is sensitive to the degree of distension, but not to compliance. Specifically, the thinner the liquid layer in the GES, the higher the rise in gastric pressure required to trigger reflux. E.g., the model predicted a gastric pressure rise >3 mmHg was required to trigger reflux when T ~ 0.05 mm, yet when T > ~0.5 mm, reflux occurred with minimal gastric pressure rise. Compliance had no effect on the probability of reflux. **Conclusions:** A potentially important distinguishing characteristic has been discovered that may partially explain the lack of direct correlation between tLESR frequency and reflux occurrence. Whereas an initially closed GES with negligible residual gastric liquid within can require several mmHg increase in gastric pressure to trigger reflux, a rather thin layer of residual liquid in a slightly distended GES can dramatically increase the probability for reflux. Taken together with the higher-than-normal basil lumen radius found in GERD patients, this model result suggests that abnormal GES distension may underlie some GERD. Support: NIH R01-DK56033.

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An endoscopic implantable device stimulates the LES on-demand by remote control in a canine model

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Background: Traditional endoscopic and surgical approaches to gastroesophageal reflux disease (GERD) therapy have focused on enhancing the mechanical barrier of the lower esophageal sphincter (LES). Selective stimulation of the LES is a more physiologic approach. We evaluated a novel endoscopic technique for on-demand stimulation of the LES with an implantable microstimulator (IMS). **Methods:** Three 30-kg dogs had routine upper endoscopy. LES pressure was recorded by esophageal manometry (EM). A mound was created with saline injection at the LES. A 30mm incision was made into the mound using a needle-knife sphincterotome. A 3.3-mm x 28-mm IMS device (Bion®) was implanted via the incision submucosally into the LES. This device is powered by an internal battery, activated via remote control, and recharged remotely through an external device. EM was repeated and post-implantation pressures were recorded with the IMS in the ''on'' and ''off'' settings. Stimulation amplitude was varied from 3 mA to 10mA with a fixed frequency of 20 Hz and pulse-width of 200 μsec. In one dog, EM was repeated for 20 minutes after deactivation of the IMS. A separate experiment was performed with implantation of a sham IMS followed by serial endoscopy, fluoroscopy, and weight measurements at weeks 1, 2, 4, and 8 to evaluate for migration. **Results:** The LES pressures prior to IMS implantation for the three dogs were 13.0 mmHg, 5.0 mmHg, and 14.9 mmHg. Following IMS placement, the LES pressures were 19.0 mmHg, 8.5 mmHg, and 4.0 mmHg. With activation of IMS at a setting of 10mA, the resultant LES pressures were 62.1 mmHg 35.1 mmHg, and 26.8 mmHg. These values were greater than *threefold* higher than the post-implantation baseline. This increase was statistically significant with a calculated p value of < 0.02. Upon deactivation of the IMS, the LES pressure declined over a

20-minute period from 26.8 mmHg to 21.4 mmHg but did not return to post-implantation baseline. The sham IMS did not demonstrate migration by fluoroscopy at weeks 1, 2, 4, and 8; however, migration was noted on necropsy at week 10.**Conclusions:** It is possible to consistently and selectively increase the LES pressure with an on-demand microstimulator. IMS implantation is a novel therapeutic approach to GERD and may have therapeutic potential for other gastrointestinal motility disorders. Further evaluation is needed to clarify the temporal relationship between LES pressure and stimulation as well as to optimize IMS placement in order to minimize migration. Additional studies are ongoing.

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The response of the esophageal body to wet and dry swallows in Chagas' disease

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Wet swallows cause a greater esophageal contraction amplitude and duration than dry swallows. In Chagas' disease there is a reduction in amplitude of esophageal contraction but we do not know if there is a difference between wet and dry swallows in the disease. We measured the area under the curve (AUC, amplitude X duration) of the esophageal contractions in 30 patients with dysphagia, positive serologic examination for Chagas' disease, radiologic slow esophageal transit, esophageal retention and distal esophageal diameter less than 4 cm, and 44 controls. The contractions were measured by the manometric method at 2, 7, 12 and 17 cm below the upper esophageal sphincter, after five swallows of a 5 ml bolus of water alternated with five dry swallows. In the control group wet swallows caused a higher AUC than dry swallows. There was no difference between wet and dry swallows in Chagas' disease patients, or between wet and dry swallows in Chagas' disease patients compared with dry swallows of the control subjects.

We conclude that in normal subjects there is adaptation of the esophageal contraction to the presence of a liquid bolus inside the esophageal body, which does not occur in patients with mild involvement of the esophagus by Chagas' disease, possibly as a consequence of the esophageal myenteric plexus impairment.

	CONTROLS		CHAGAS' DISEASE	
	WET	DRY	WET	DRY
2 cm	102.4±7.9*	81.7±7.4	86.7±10.6	81.3±7.8
7 cm	75.6±9.4*	54.1±6.0	58.6±6.7	49.7±6.3
12 cm	155.7±13.7*ϕ	81.8±10.4	68.0±7.3	65.4±7.0
17 cm	211.6±17.3*ϕ	99.7±12.2	93.0±12.4	80.5±12.7

*mean ± SEM (mmHg x s) * p < 0.04 vs controls dry *
ϕp < 0.01 vs Chagas wet and dry.

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Oral and pharyngeal transit of a paste bolus in Chagas' disease

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Previous studies have reported that swallowing of a liquid bolus by Chagas' disease patients cause a longer oral phase, longer pharyngeal clearance and a longer interval between the onset of pharyngeal and proximal esophageal contractions than in normal subjects. In this study we measured by the scintigraphic method the oral and pharyngeal transit of a paste bolus in 20 patients with a serologic, radiologic and manometric diagnosis of esophageal involvement by Chagas' disease and 21 control subjects. Each subject performed in the sitting position a swallow of a 10 ml paste bolus (50 ml of water with 4.5 g of instant food thickener) labeled with 55.MBq of 99^{m} technetium phytate. After the scintigraphic registration regions of interest (ROI) corresponding to mouth, pharynx and proximal esophagus were delineated. Time activity curves were generated for each ROI. There was no difference between patients with Chagas' disease and control subjects in the duration of oral and pharyngeal transit, amount of pharyngeal residue or in flux of bolus entry in the proximal esophagus (Controls: 13.28 ± 7.06 ml/s, Chagas: 13.37 ± 4.07 ml/s). Patients with Chagas' disease had a higher amount of oral residue and a longer pharyngeal clearance duration than control subjects.

We conclude that, when swallowing a paste bolus patients with Chagas' disease may have an increase amount of oral residue and a longer pharyngeal clearance duration than asymptomatic volunteers.

	ORAL		PHARYNGEAL		
	Transit (s)	Residues (ml)	Transit (s)	Clearance (s)	Residues (ml)
Control	0.70±0.35	0.69±0.82*	0.63±0.42	0.73±0.40*	0.81±0.49
Chagas	0.64±0.22	1.12±1.25	0.49±0.12	0.86±0.20	0.71±0.51

mean ± SD * p < 0.05 vs Chagas.

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Critical role of stress in acid-pepsin induced esophageal mucosa dilated intercellular spaces (DIS) and increased permeability

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In patients with non-erosive GERD, heartburn is thought to occur when acid reaches sensory nerve endings through mucosal dilated intercellular spaces (DIS). Stress or anxiety may increase heartburn perception. Visceral hyperalgesia due to central hypersensitivity is currently the most accepted hypothesis for stress-increased esophageal symptoms. In the rat, acute stress increases gastric and intestinal mucosa permeability. We hypothesized that stress might also increase esophageal mucosa permeability and thereby, enhances reflux induced esophageal symptoms.

Aim: To evaluate the effect of stress on esophageal mucosa permeability and diameter of intercellular spaces in rats. **Methods:** Male Sprague-Dawley rats were submitted to partial retraint stress (PRS) for 2h, followed by 2h of free movements in their home cage. Control rats were maintained in their home cage for 4h. Segments from esophageal mucosa (15x8mm) from these animals were mounted in diffusion chambers. Mucosal permeability was assessed by measuring the luminal-to-serosa passage of ^{51}Cr-EDTA (392 Da), FITC-dextran 4000 Da (FD4) and 20000 Da (FD20). The tissue was pre-incubated for 30 min with Krebs pH 7.2 or HCl pH 2.0 + pepsin 1 mg/mL (acid-pepsin) in the luminal side. Permeability was expressed in pmols/cm^2 and nmols/cm^2 of ^{51}Cr-EDTA and FD respectively, obtained at the serosal side. Diameter of intercellular spaces was assessed using H-E staining and transmission electron microscopy (TEM). **Results:** The stress protocol was effective: PRS increased the fecal output (4.14±0.7 vs. 10±1.7, P<0.01), small intestinal permeability to FD20 (P<0.0001) and plasmatic glucose levels by 24±5.4 % (P<0.01). Acid-pepsin exposure alone, didn't modify the esophageal mucosa permeability to the tested molecules., Stress, by itself, increased permeability for the smallest molecule (P<0.001). In contrast with the lack of effect of acid-pepsin alone, exposure of mucosa from stressed rats to acid-pepsin resulted in increased permeability for the smallest molecule (P<0.001). In contrast with the lack of effect of acid-pepsin alone, exposure of mucosa from stressed rats to acid-pepsin resulted in increased permeability to all molecules tested ^{51}Cr-EDTA (p=0.016), FD4, (P<0.0001) and FD20 (P<0.0001). Light and TEM microscopy showed DIS only in the esophageal mucosa of stressed rats exposed to acid-pepsin. **Conclusions:** In rats, acute stress increases esophageal mucosa permeability. Exposure to esophageal mucosa to acid-pepsin induces DIS and increases permeability only in previously stressed rats. These results suggest that stress plays a critical role in the acid-pepsin-induced DIS and increased esophageal mucosa permeability. We propose that this mechanism may contribute to stress-influences on esophageal symptoms in man.

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Health-related quality of life and symptom scores in clinically adequately treated achalasia patients: A cross-sectional study

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Introduction: To date there is limited knowledge concerning symptom characteristics of achalasia patients after treatment and their effect on the health-related quality of life (HRQoL). Furthermore, the prevalence of functional dyspepsia-like (FD) and irritable bowel syndrome-like (IBS) symptoms in patients with achalasia is unknown. **Aim:** To investigate the frequency and severity of esophageal symptoms and their relation to HRQoL. Secondly, the prevalence of FD and IBS in treated achalasia patients. **Methods:** A cross-sectional survey was conducted among 171 treated patients with achalasia, who visited our clinic between 2000–2005. Patients were approached by mail. The frequency of dysphagia, regurgitation (none, occasionally, daily or several times a day) and weight loss (none, <5kg, 5–10kg, >10kg) were each scored on a 0–3 scale. Chest pain was scored on a 0–4 scale (none, <monthly, monthly, weekly, daily) (Eckardt et al.). SF-36 and a recently developed disease-specific measure (Urbach et al.) were used to measure HRQoL. A customized questionnaire assessed the Rome II criteria regarding FD and IBS symptoms. **Results:** 126 patients responded (76.6%, mean age 48 yr (39.3–61.8), 54.7% men). Patients were treated with a series of three pneumatic dilations (n=72) and/or Heller myotomy (n=39/n=7, respectively) or botox (n=8). The prevalences of dysphagia ≥ once daily or chest pain ≥ once a week were 46% (n=58) and 38% (n=48) respectively. Thirty-two patients (25.4%) complained of both chest pain ≥ once a week and dysphagia ≥ once daily. Achalasia patients scored lower than 118 age- and sex- matched healthy controls on all the SF-36 subscale scores (all P<0.001), except health change. Patients with chest pain ≥ once a week and dysphagia ≥ once daily scored lower than patients with less frequent symptoms on the SF-36 subscales social functioning (P=0.003), pain (P<0.001) and general health (P=0.002) as well on the disease specific HRQoL outcome (P<0.001). Prevalences of FD (19%) and IBS (17.5%) were 4 respectively 1.5 times higher than in the general Dutch population. Achalasia patients with chest pain ≥ once a week showed a higher probability to fulfill FD and/or IBS criteria (adjusted OR 3.35 [1.4–8.1]) **Conclusion:** Many achalasia patients remain severely symptomatic after treatment and have decreased HRQoL. In patients with frequent symptoms lower general and disease-specific HRQoL was observed compared to patients with less frequent symptoms. Achalasia patients with frequent chest pain have a higher probability to fulfill FD and/or IBS criteria.

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Relationship between upper gastrointestinal symptoms and positive 24 hr esophageal pH tests in Thai patients with chronic upper gastrointestinal symptoms

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To study the relationship between upper gastrointestinal (UGI) symptoms and positive esophageal pH tests in Thai patients with chronic UGI symptoms. **Methods:** 132 patients (44 M, age 45 ± 14 yr) with chronic UGI symptoms [16 GERD, 58 reflux like dyspepsia (RD), 58 functional dyspepsia (FD)] were included. Each underwent a 24-hr esophageal pH testing after an esophageal manometry. All patients were questioned about their UGI symptoms (heartburn, acid regurgitation, dysphagia, epigastrium burning, upper abdominal discomfort, upper abdominal pain, chest pain, belching, nausea, vomiting, anorexia, early satiety, and bloating) using a symptom questionnaire. A positive pH test was defined as % time pH<4 in the lower esophagus >4.5%. **Results:** 67 patients had positive pH tests. There was no difference of the distribution of positive pH tests among GERD (n=8), RD (28), and FD(31)(p>0.05). There was no difference of the BMI and gender distribution between patients with positive and negative pH tests (p>0.05). Patients with positive pH tests had higher prevalence of acid regurgitation symptom than patients with negative test (p<0.01). There was no difference of the prevalence of heartburn, dysphagia, chest pain, belching, upper abdominal pain, upper abdominal discomfort, anorexia, early satiety, nausea, vomiting, bloating, and epigastrium burning between patients with positive and negative pH test (p>0.05). **Conclusion:** Acid regurgitation was associated with positive 24 hr esophageal pH tests in Thai patients with chronic UGI symptoms, whereas heartburn and other UGI symptoms were not associated. This study suggests that GERD symptoms in Thai patients may be different from those of Western countries.

Symptom (N: S+/S–)	pH neg (N: S+/S–)	pH pos (N: S+/S–)	Symptom (N: S+/S–)	pH neg (N: S+/S–)	pH pos (N: S+/S–)
Heartburn (69/63)	35/30	34/33	Anorexia (44/88)	26/39	18/49
Acid regur (90/42)	37/28	53/14*	Early satiety (74/57)	35/29	39/28
Dysphagia (31/101)	14/51	17/50	Nausea (70/62)	32/33	38/29
Chest pain (53/79)	25/40	28/39	Vomiting (31/100)	14/50	17/50
Belching (91/15)	45/8	46/7	Bloating (107/25)	54/11	53/14
Upper abd pain (68/64)	39/26	29/38	Epigastrium burning (90/42)	43/22	47/20
Upper abd discomfort (91/41)	47/18	44/23			

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Distension-induced sensory responses in the esophagus of healthy humans are due to mechanical rather than ischemic mechanisms

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Functional chest pain of presumed esophageal origin is common in the population but its pathophysiology is essentially unknown. Esophageal distension evokes pain in patients and healthy volunteers. Regional restricted blood flow due to distension as well as mechanical stretch can be the mechanism for the pain response. The purpose of this study was to evaluate pain responses in healthy volunteers to learn more about pain mechanisms. We have previously developed a multimodal catheter device which combines bag distension and laser Doppler flowmetry, manometry, HF intraluminal ultrasound, and evaluation of symptoms using a VAS. Using the same device in the present study, ramp distension at an infusion/deflation rate of 10 ml/min was repeated several times up to VAS 7. After the fourth distension 20–40mg of butylscopolamine was injected intravenously for relaxation of esophageal smooth muscle. The mucosal perfusion was evaluated at VAS 1 (first sensation), VAS 5 (pain threshold) and VAS 7 (moderate pain). The bag pressure was measured and ultrasonographic images were analyzed for evaluation of the mechanical stimulus. Data from 22 volunteers were analyzed (14F/8M, mean age 43.6 (range 24–67) years. The decline in perfusion units (PU) from VAS1–5 and 1–7 was 17% and 26% without butylscopolamine. With butylscopolamine the decline in PU was 7% and 15%. We observed episodes of powerful contractions using ultrasonography and corresponding decrease in PU. During these contractions the VAS level seldom reached five or higher, unless there was multiple repetitive powerful phasic contractions over a period of time (>30 sec), or unless the phasic contraction had a long duration or came late in the infusion phase. The non-pain and pain responses were closer associated with the mechanical stimuli than with the changes in perfusion. In conclusion, sensations in the non-painful and painful ranges are more closely related to the mechanical stimulus than to the ischemic response. Future studies will evaluate patients with functional chest pain to clarify pain mechanisms in these patients.

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Diagnosis of non-acid gastroesophageal reflux (GER) using ambulatory multi-channel intraluminal impedance and pH (MIIpH) measurement

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Background: Some patients with symptoms of GER and poor response to acid suppressant and/or normal 24h oesophageal pH profiles, may have non-acid reflux. MIIpH measurement is able to detect acid and non-acid GER using pH and impedance sensors. The aim of this study was to evaluate the diagnostic value of MIIpH measurements in a clinical setting to detect non-acid GER in patients with symptoms but with normal pH profile. **Method:** Forty four patients (24 female, age 23 –67) with GER symptoms who had normal 24h oesophageal pH measurements underwent 24h MIIpH measurements using a portable data logger (Sleuth System, Sandhill Scientific, USA). All patients had oesophageal manometry prior to the measurements. The data was analysed by computer assisted manual analysis using dedicated software (Bioview, Sandhill Scientific, USA). All reflux treatments were withdrawn 5 days prior the measurements and patients were allowed normal but documented food and drink. **Results:** Twenty four patients had a pathological level of non-acid GER. The median number of non-acid reflux episodes was 13 per study (range 3–60). Mixed (liquid and gas) reflux episodes (median 23 per study, range 1 – 51) were more frequent than liquid only reflux episodes (median 12 per study, range 1 – 54). The median bolus clearance time was 13 seconds (range 3 – 24). **Conclusions:** This study has shown that 55% of the patients with GER like symptoms, who have normal pH study, had non-acid GER. MIIpH measurement is a useful diagnostic tool to detect acid and non-acid GER in a clinical setting and is used more frequently in patients who have symptom suggestive of non-acid reflux, that is, excessive belching, early satiety and gastric bloating.

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Impaired gastric motility and its relationship to reflux symptoms in patients with nonerosive gastroesophageal reflux disease

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Background: More than half of patients with reflux-related symptoms such as heartburn have no endoscopic evidence of mucosal breaks, which is called nonerosive gastroesophageal reflux disease (NERD). The response to proton-pump-inhibitor treatment is lower for NERD patients than for those with erosive gastroesophageal reflux disease. The pathogenesis of NERD, which is thought to differ from erosive esophagitis, may be multifactorial. The role played by gastric motility in symptom generation in patients with NERD has not been examined. **Aims:** We aimed to elucidate the gastric motility in patients with NERD and the efficacy of a prokinetic agent in the treatment of NERD. **Methods:** Gastric motility was evaluated with cutaneously recorded electrogastrographs (EGGs) and by measurement of gastric emptying using the acetaminophen method in 20 patients with NERD and in 11 matched healthy controls. We diagnosed NERD based on evidence of the patients having heartburn at least 2 days a week and no erosive esophagitis on endoscopy. NERD patients were treated with a prokinetic agent (15mg of mosapride, a 5-HT_4 receptor agonist, orally three times daily) for a period of 4 weeks, after which gastric motility was measured again. The global therapeutic outcome was rated as markedly improved, slightly improved, unchanged, or deteriorated. **Results:** Compared to the healthy controls, the NERD patients had a significantly lower percentage of normogastria in both the fasting and postprandial states, a lower power ratio in EGGs, and a lower concentration of plasma acetaminophen, indicating delayed gastric emptying. Eight patients had normal gastric motor function (Group A), and 13 patients showed abnormalities of either gastric myoelectrical activity or gastric emptying (Group B). After 4 weeks of treatment with mosapride, gastric myoelectrical activity and gastric emptying improved significantly in patients with both Group A and Group B compared to pretreatment. The global response to treatment was markedly or slightly improved in 14.3% of Group A patients versus 46.2% of Group B patients ($p<0.05$). **Conclusions:** Sixty-five percent of patients with NERD showed gastric hypomotility including impaired gastric myoelectrical activity and delayed gastric emptying. Gastric hypomotility appears to be an important factor in the generation of reflux symptoms in some NERD patients.

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Clinical utility of esophageal manometry

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Background: While esophageal manometry (EM) is commonly ordered to evaluate patients (Pts) with dysphagia, chest pain (CP) and gastroesophageal reflux disease (GERD), the clinical utility of EM is not known. **Methods:** Prior to performing EM, referring physicians (GIs, surgeons, primary care) prospectively completed a questionnaire requesting: indications for the test (primary and secondary); symptoms (primary and secondary; duration); previous testing for the problem; and medication use. Demographic information was obtained from the patient. Two weeks after the referring physician received EM test results, a follow-up questionnaire was sent asking whether EM provided new information that either changed the Pt's diagnosis or management plan. Results of EM were classified using standard criteria. **Results:** During a 12 month period 569 EMs were performed and 444 were available for inclusion; 286 fully completed questionnaires were returned (64.4%) and are the basis for this analysis. The mean age (±SD) at time of manometry was 52 (± 15 years); 58% were women. The most common reason for requesting EM was to accurately place a pH probe or Bravo capsule (34%); evaluation of dysphagia (30%), chest pain (12%), and acid reflux symptoms (11%) were somewhat less common. There was a significant difference found among referring physicians, NPs, PAs, and the primary reason for referral, with GIs more likely than surgeons to refer for CP, dysphagia, and to rule out a motility disorder ($p < .0001$). Procedural evaluation prior to EM included EGD (73%), barium swallow (42%), chest x-ray (24%) or an abdominal x-ray (14%). Overall, 63% of EM were abnormal; 81% in GERD Pts, 71% in dysphagia, and 59% in CP. Physicians, NPs, PAs, reported that new information was obtained in 100% of cases of GERD, 79% of dysphagia, and 73% of CP. Results of EM changed the Pts diagnosis 22% of the time in GERD, 47% in dysphagia, and 38% in CP. EM results led to a change in Pt management in 50%, 65% and 53% in those with GERD, dysphagia, and CP respectively. GI physicians were more likely to change their management based on results of testing than surgeons, IM, or NPs/PAs ($p = 0.04$). **Conclusions:** EM is a clinically useful test as it frequently provides new information and leads to a change in Pt diagnosis or management. It is most helpful in patients with the primary complaint of dysphagia or chest pain.

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The role of $GABA_A$ receptors in the control of transient lower esophageal sphincter relaxations (TLESRs) in the dog

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TLESRs are triggered by activation of mechanosensitive gastric vagal afferents and are the major cause of gastroesophageal reflux and therefore an important target for therapeutic intervention in GERD. Activation of the metabotropic receptor for GABA, the $GABA_B$ receptor, has been shown to inhibit TLESRs. The aim of the present study was to assess the role of the ionotropic $GABA_A$ receptor in the regulation of TLESR. TLESRs were quantified using Dentsleeve manometry in dogs, and $GABA_A$ agonists were dosed i.v. prior to

gastric distension at 10 mm Hg. Immunohistochemistry and PCR were used to localize $GABA_A$ receptors in the nodose ganglion, the source of vagal afferents. The prototypical $GABA_A$ agonist muscimol produced an inhibition of TLESRs ranging from 19 ± 8% at 0.082 μmol kg^{-1} to 56 ± 17% at 8.2 μmol kg^{-1} (n=6 for all doses). The two other $GABA_A$ agonists evaluated, isoguvacine (8.2 μmol kg^{-1}) and THIP (5.7 μmol kg^{-1}), as well as the $GABA_A$ positive allosteric modulator diazepam (3.5 μmol kg^{-1}), had no major effects on TLESRs at the doses tested (THIP 15 ± 9% inhibition; isoguvacine 10 ± 10% stimulation; diazepam 29 ± 12% inhibition) which were limited by emesis (THIP and isoguvacine) or restlessness/sedation (diazepam). Of the predominant $GABA_A$ subunits (the α, β and γ isoforms), α and β but not γ were detected in the dog nodose ganglion by immunohistochemistry and PCR, raising the possibility that the effects of muscimol may not be restricted to central $GABA_A$ receptors. The present observations demonstrate that $GABA_A$ receptors exert an inhibitory control of TLESRs. The results warrant further studies using $GABA_A$ isoform-selective agonists to define the identity of receptors involved. This work was in part funded by AstraZeneca.

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GERD-related alterations to the tonic intrinsic lower esophageal sphincter in the resting state, and to the stiffness of relaxed esophageal muscle during simulated tLESR

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The gastro-esophageal segment (GES) has the dual role of maintaining tone in the resting state and controlling opening in the absence of tone (tLESR). Using pharmacologic separation of the smooth muscle (SM) and skeletal muscle components, we have proposed the existence of two SM components, one intrinsic to the esophageal wall overlapping the crural sphincter, and one maintained by the sling-clasp muscle groups at the esophago-cardiac junction. Here we show relationship between the tonic and relaxed states with GERD. **Methods:** A high-frequency endoluminal ultrasound (US) and simultaneous manometry catheter was used to evaluate normal volunteers (NV) and patients with symptomatic GERD without hiatal hernia, during steady pull-through from the stomach through the GES. (I) Pull-throughs were performed in the resting state before and after intravenous administration of atropine in 15 NVs and 7 GERD patients. Pressures were referenced to intragastric pressure. Post-atropine pressures were subtracted from pre-atropine pressures after referencing spatially to the lower margin of the crural diaphragm (CD) obtained from the ultrasound images. (II) Pull-throughs were performed during deglutitive inhibition with bolus swallows as a physiological model of opening in the absence of tone (tLESR). Using a knowledge-based image analysis system, cross-sectional lumen and wall geometries were quantified during opening in 6 NVs and in 6 patients. **Results:** In the NVs the difference between the pre- and post-atropine pressures profiles (intrinsic atropine-sensitive sphincter components) displayed distinct proximal and distal peaks. In the GERD patients the distal peak of the subtraction curves was fully absent in all patients, while the proximal peak was present and in the same axial position relative to the CD as in the NVs. (II) As compared to the NVs, the average radius to the inner smooth muscle was significantly greater in the GERD group by ~2 mm, both in the resting state and during opening (max radius 7.10 mm ± 0.53SD vs. 9.16 mm ± 2.9SD). After opening, the effective stiffness of the GES was ~6 times lower in the GERD group as compared to NVs. **Conclusions:** The distal component to the intrinsic sphincter seen in NVs is missing in the GERD patients. Furthermore, the GES is more distended and more compliant in patients with GERD. We hypothesize that gastric sling fiber/clasp fiber complex tends to be defective in patients with GERD, leading both to an absence of tone at the esophago-cardiac junction and a more distended and less stiff GES during tLESR. Defective sling-clasp muscles would both reduce protection in the resting state and increase the probability of reflux during transient periods of relaxation. These findings may be important to the pathophysiology of reflux disease. Funding: NIH R01 DK59500, C. R. Bard Inc.

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Endoscopic plication partially restores a missing tonic pressure component to the LES and the stiffness of relaxed esophageal muscle during tLESR in patients with GERD

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In previous studies using pharmacologic separation of smooth and skeletal sphincteric components of the gastro-esophageal segment (GES), we (1) argued for the existence of a proximal intrinsic sphincter component superposed with the crural diaphragm (CD), and a distal component associated with sling-clasp muscles at the gastro-cardiac junction, and (2) showed that the lower component is missing in a group of GERD patients. We further showed that the GES with GERD is more distended and more compliant. Here we analyze the consequences of endoscopic plication (EP) on the GERD-related alterations. **Methods:** 10 GERD patients were evaluated during breath hold under full inspiration (FI) and full expiration (FE) with pull-throughs of a high-frequency ultrasound (US)-manometry catheter assembly from the stomach through the GES, before and after EP (Endocinch). Pull-throughs were repeated after intravenous atropine. Pressures were referenced to gastric. Post-atropine pressures were subtracted from pre-atropine pressures referenced to the CD. US images were analyzed for plication configuration and location, suture depth, and pressure changes. Cross-sectional GES geometries and pressures were quantified during swallow-induced bolus distension in 6 normal controls and 6 patients, pre and post EP. Lumen radii, areas, and muscle thicknesses were quantified and wall tension was calculated from a force balance. **Results:** 8 of 10 patients had clinical benefit from EP. The majority of sutures were localized within the submucosa, and most plications were at or just below the CD. The distal peak in the atropine-sensitive subtracted pressure profiles, previously associated with the sling-clasp fibers at the esophago-cardiac junction and missing in GERD patients, was partially reestablished in the patient group at approximately the same location as in normal volunteers. The distal portion of the pressure profile was lengthened in the area of the plications. The stiffness of the relaxed GES, approximately 6 times lower in the GERD group, increased to above normal values after EP, but only after already opening. The plications did not delay luminal opening, but maximum luminal opening was restricted post EP. **Conclusions:** The plications partially restored a portion of the smooth-muscle sphincter missing in the GERD patients, improving protection against reflux in the resting state. Stiffness of the relaxed GES was enhanced by the plications, thereby reducing maximum luminal opening. However, the enhancement occurred too late to suppress the occurrence of opening during tLESR. We hypothesize that the EP worked primarily by augmenting an absent distal tonic component and increasing the stiffness of this area. Further improvement may be possible by restricting opening during tLESR. Funding: NIH R01 DK59500; CR Bard, Inc.

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Axial stretch of the esophagus induces lower esophageal sphincter relaxation

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Introduction: Swallow-induced and esophageal-distension induced relaxation of the lower esophageal sphincter (LES) are associated with its cranial movement, of up to 20 mm, due to a concurrent longitudinal muscle contraction. The latter causes an axial stretch on the LES. We tested the effects of a cranially directed mechanical stretch on the LES pressure. **Methods:** Studies were conducted in 7 opossum, a silicon tube was placed via mouth into the esophagus and surgical laprotomy was performed. Two needles with silk sutures were passed, 90 degrees apart, through both esophageal walls and silicon tube, 1–2 cm above the LES. Tube was withdrawn by mouth and one end of each of the 4 sutures was anchored to the esophageal wall and the other end exerted graded cranial stretch on the LES. The LES pressure was monitored using a sleeve sensor.

Effect of nitric oxide antagonist (L-NNA 20mg/kg, intravenous bolus,) was also studied. **Results:** Axial stretch caused LES relaxation with the recovery of LES pressure following cessation of the stretch. The degree of LES relaxation was directly related to the applied weight. The weights required to induce maximal LES relaxation differed among animals (714±348gms). A cranial movement of 15–20 mm of the esophagus occurred with the weight that induced maximal LES relaxation. Increasing the duration of axial stretch increased the duration of LES relaxation. Following L-NNA, a nitric oxide inhibitor administration, the maximal LES relaxation in response to axial stretch was reduced from 79±12% o 23±18%, and the residual pressure was increased from 10±6 mmHg to 45±15mmHg. **Conclusions:** Our data support the presence of an axial stretch sensitive inhibitory pathway in the opossum LES. The role of stretch activated neuron in the mediation of swallow-induced and esophageal distension induced LES relaxation requires further exploration.

Table: Axial Stretch-induced LES relaxation (Mean ± SEM)

Weight	Before L-NNA	After L-NNA
200 gm	37±9%	13±18%
400 gm	55±11%	13±6%
600 gm	62±8%	17±9%
800 gm	66±6%	14±4%
1000 gm	67±8%	17±6%
1200 gm	87±2%	19±4%

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Novel method of chromoendoscopy with Congo red in the detection of GERD

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Introduction: Erosive esophagitis in patients with reflux symptoms is highly specific for the diagnosis of gastroesophageal reflux disease. However, in 35–70%, the endoscopic examination may be normal. Congo red is a pH indicator that changes from red to dark blue or black in acidic conditions. It is a basic reactive dye with the capacity to visualize acid-producing gastric mucosa endoscopically. Apparently, it can be used with endoscopy to diagnose nonerosive reflux disease. However, there is no works studying chromoendoscopy of esophagus with Congo red. **Methods:** A total of 22 patients with reflux symptoms occurring at least 3 episodes per week and demonstrated no esophageal mucosal injury on endoscopy were enrolled. Excluded were those patients with intake of H2 receptor antagonist or proton pump inhibitor within the past 7 days and GI bleeding. All endoscopic procedures were performed by a single endoscopyst with identical equipment. After obtaining permission, we used to spray 2 ml 0,5% Congo red solution onto the esophageal mucosa. The tip of catheter has been located 4–5 sm above from Z-line. We estimated the color shift in the esophagus during half minute. **Results:** The color shift of Congo red was classified into:

Type 1 - has not occurred the color shift to surface of the esophagus during 30 seconds,

Type 2 – reflux of blue/black dye from gastric cardia to esophageal lumen in 10–30 seconds after staining,

Type 3 - reflux of blue/black dye from gastric cardia to esophageal lumen in 2–10 seconds after staining,

Type 4 - the color shift, which has occurred immediately after spraying of Congo red onto the esophageal mucosa.

In all 22 cases color shift we have estimated as: type 1 – 5, type 2 – 6, type 3 – 7, type 4 – 4 patients. In 3 cases we observed reflux of gastric content into esophagus, but did not observe color shift of Congo red. **Conclusions:** Chromoendoscopy with Congo red is a simple, fast, inexpensive, and well-tolerated method. It requires the further studying for detection of its effectiveness in the diagnosis of gastroesophageal reflux disease.

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Impact of nadir lower esophageal sphincter pressure on the relationship between wave amplitude and esophageal bolus clearance

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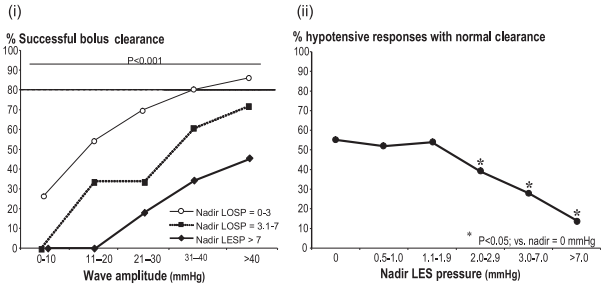
Effective esophageal bolus clearance (EBC) independently depends on (i) the strength of the peristaltic contraction and (ii) the flow resistance across the lower esophageal sphincter (LES). The relationship between these two factors and its effects on EBC, however, is unclear. **Aim:** To assess the relationship between nadir LES pressure and wave amplitude on EBC. **Methods:** Concurrent esophageal manometry and multi-channel intra-luminal impedance was performed in 142 subjects (42=healthy, 38=non-obstructive dysphagia and 62=gastroesophageal reflux). In each subject, esophageal motility and clearance was measured concurrently at 4 sites, each 5-cm apart starting 2cm above the LES. Ten, 5-ml liquid boluses and 10 x 5-ml viscous boluses were tested in each subject. Swallow responses were categorized by nadir LES pressure. For each category of nadir pressure, distal esophageal wave amplitude was binned into groups of 10mmHg and the % of successful EBC in each wave amplitude bin was determined **Results:** Impaired EBC present in 28% of liquid and 32% of viscous responses. Responses with failed clearance had significantly lower pressure wave amplitude at all sites, higher basal (8.9±0.7 vs. 6.4±0.3 mmHg; P<0.0001) and nadir LES pressure (2.7±0.4 vs. 1.0±0.1mmHg, P<0.001). For both liquid and viscous boluses, nadir LES pressure significantly altered the relationship between pressure amplitude and bolus clearance and shifted the curves to the right (fig (i)). Hypotensive responses were less likely to clear either liquid or viscous boluses once nadir LES pressure ≥ 2mmHg (fig (ii)). **Conclusions:** Nadir LES pressure has a significant impact on the relationship between pressure wave amplitude and esophageal bolus clearance, even at the upper range of normal. This impact may be clinically important in patients with ineffective esophageal dysmotility.



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Can esophageal impedance predict the occurrence of post-fundoplication dysphagia?

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Dysphagia is a significant sequela of fundoplication and may be related to impaired esophageal clearance. Pre-operative assessment of clearance by manometry and/or fluoroscopy is suboptimal and fails to predict the occurrence of post-fundoplication dysphagia. Multi-channel intra-luminal impedance (MII) measures esophageal clearance directly. **Aim:** To assess the impact of laparoscopic fundoplication on esophageal clearance and its relationship to dysphagia, using combined manometry-MII. **Methods:** 11 patients with proven reflux disease (6M:5F; 54 ± 4yrs) underwent combined manometry-MII and symptom assessment before and 3–6 months after laparoscopic fundoplication. Esophageal motility and impedance were measured concurrently at 4 sites 5-cm apart starting 2cm above the lower esophageal sphincter

(LES). Ten, 5-ml liquid (L) boluses and 10 x 5-ml viscous (V) boluses were tested in each subject. Impaired clearance was defined by either prolonged bolus presence time (BPT) and/or total bolus transit time (TBTT). An individual was judged to have abnormal esophageal clearance function if there were > 2 liquid or > 3 viscous responses with impaired clearance. **Results:** Fundoplication increased nadir LES pressure, distal esophageal wave amplitude, and distal and total propagation time (P<0.05). Fundoplication increased the proportion of responses with impaired clearance (liquid: 60/110 vs. 32/110, P=0.001; viscous: 46/110 vs. 17/110, P<0.001), and increased BPT in the mid and distal esophagus and TBTT. After fundoplication, more patients had abnormal bolus clearance (9/11 vs. 4/11) and the median number of impaired responses per patient increased (L: 5(2.5–8) vs. 2(0.5–3.5), P=0.07; V: 4(2–6) vs. 1(0–2.5), P=0.03). No patient had improved bolus clearance post-operatively. There was a positive correlation between the nadir LESP and BPT at the most distal segment (r=0.2–0.4, P=0.01). Pre-operative impaired esophageal bolus clearance failed to predict the presence of dysphagia, both pre-operatively (3/5 patients with impaired clearance vs. 3/6 patients with normal clearance) and post-operatively (3/5 patients with impaired clearance vs. 4/6 patients with normal clearance). **Conclusions:** Fundoplication impairs esophageal clearance, predominantly from the distal 2cm of the esophagus. This may be related to increased resistance to flow at the gastroesophageal junction caused by the wrap. The relationship between impaired bolus clearance and dysphagia is poor, suggesting abnormal esophageal sssensation may be a more important factor.

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Morphometric evaluation of muscle and neuronal innervation of esophageal wall in patients with nutcracker esophagus and ineffective esophageal motility

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Backgrounds/Aims: Nutcracker esophagus (NE) is characterized by high-amplitude peristaltic contractions in the distal esophagus and ineffective esophageal motility (IEM) is defined as low amplitude or nontransmitted contractions in the distal esophagus. While their pathogenesis are not known, and no study has been reported on neuronal innervation of the esophageal wall. We previously reported a preliminary study of neuronal innervation of esophageal wall in patients with NE and IEM by immunohistochemical study, which showed densities of choline acetyltransferase (ChAT) in the wall were significantly increased in patients with NE. Densities of neuronal nitric oxide synthase (nNOS) in myenteric plexus (MP) and circular muscle (CM) layer were significantly increased in IEM. Therefore, we undertook this study to validate our previous study and investigate a morphometric evaluation of muscular thickness and fibrosis of esophageal wall. **Methods:** Twenty two consecutive patients with non-obstructive stomach cancer were enrolled. Before total gastrectomy, EGD and esophageal manometry was performed and classified them into NE (n=10), IEM (n=6), and normal motility (n=6). A full-thickness biopsy specimen was taken from the distal esophagus. The hematoxylin-eosin, Masson's trichrome along with immunohistochemical study for PGP-9.5, neuronal cell marker, ChAT, marker of cholinergic neurones, and nNOS, marker of nitrergic neurons in MP and CM layer were performed and calculated their distribution using an image analyzer. The thickness of inner CM layer was measured in millimeters. **Results:** The thickness and fibrosis in CM layer were not differ significantly among 3 groups. The density of PGP-9.5 reactive neuronal structures were not changed significantly both in MP and CM of NE. However, densities of ChAT both in MP and CM layer were significantly increased in patients with NE. Densities of nNOS in MP and CM layer were significantly increased in IEM. **Conclusions:** Nutcracker esophagus and ineffective esophageal motility may relevant with an imbalance between the excitatory and inhibitory elements of the esophageal innervation.

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Outcome of pneumatic dilation on esophageal function and morphology using high-frequency intraluminal ultrasound in patients with achalasia

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Background: Achalasia is a well-defined primary esophageal motility disorder for which pneumatic dilation is an established therapeutic method. Although it is known that any treatment can little restore the muscular activity of the denervated esophagus, we have experienced a few cases which exhibited the recovery of peristalsis after pneumatic dilation. High-frequency intraluminal ultrasound (HFIUS) is considered to be the effective imaging technique, and recently, there have been a few attempts to evaluate esophageal sensory and motor function using HFIUS. An increase in esophageal muscle thickness using HFIUS has been reported to be an important feature of primary esophageal motility disorder such as achalasia. The aim of this study was to establish the outcome of pneumatic dilation with regard to the esophageal function and morphology using HFIUS as well as the common methods including the clinical, radiological and manometric features. **Methods:** Twenty-two patients (eleven men and eleven women, mean age 40 ± 15 years) with achalasia treated by pneumatic dilation were enrolled in this study. The assessment of the clinical and symptomatic improvement, the maximal diameter of esophageal body on barium esophagogram, the lower esophageal sphincter (LES) resting pressure on manometry, and the esophageal mean muscle thickness and cross sectional area (CSA) at 3 cm above LES on HFIUS was performed for baseline assessment and was done 6 months later after pneumatic dilation. **Results:** Enrolled patients exhibited distinctly different features after pneumatic dilation comparing before pneumatic dilation; a recovery of weight, an improvement of dysphasia, a reduction of maximal diameter of the esophageal body, and a decrease of LES resting pressure. These differences between before and after pneumatic dilation were statistically significant. Esophageal muscle thickness and CSA were observed to be significantly decreased after pneumatic dilation at 3 cm above LES. **Conclusions:** Esophageal muscle thickness and CSA were restored after pneumatic dilation in this study, as were the clinical, radiological, and manometric features in patients with achalasia.

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MR fluoroscopy in the assessment of esophageal motility disorders

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Aim To evaluate whether MR-fluoroscopy withTurbo-FLASH sequences (MR-F-TFL) can properly assess esophageal motor function. **Methods and materials** Fourteen endoscopy-negative dysphagic patients with manometric diagnosis of achalasia (n=8 mean age 58 yrs ±12.9) and of Non Specific Motility Disorders (NSMD); (n=6 mean age 60.5±14.4 years), and asymptomatic subjects (n=7 mean age 27.5±1.7 years) were studied using a 1.5 T magnet (Magnetom Avanto: Siemens, Erlangen, Germany) equipped with phased-array coil. Imaging protocol was based on the employment of Turbo-FLASH sequences on sagittal, coronal and axial planes to achieve motility evaluation (TR 600 mS; TE 1,3 mS; Flip Angle 8°; Thickness 20mm; FoV 350; Matrix 128x256; N. acquisition 120; TA=50 sec) during oral administration of positive contrast agent (yoghurt+Gd-DTPA 0.5 M, 1:100 boluses). **Results** MR-F-TFL showed a) in normal subjects: the presence of propagated (2.6 cm/sec) after swallow contractions (primary peristalsis), absence of non-propagated contractions, esophageal transit time 8.6±1.1 sec, coordinated opening of the cardias with after-swallow propagated contractions; b) in achalasic patients: propagated contractions in one case only, the presence of non-propagated contractions, esophageal transit time 44.4±18.0 sec (p<0.05 vs normal), occasional or absent coordinated opening of the cardias, c) in patients with NSMD: propagated contractions in two cases, non-propagated contractions in

all but one patient, esophageal transit time of 22±19.3 sec (p<0.05 vs achalasic), occasional or constant coordinated opening of the cardias in all but one patient.**Conclusion** R-F-TFL showed that esophageal motor abnormalities are associated with delayed esophageal transit, significantly more in patients with achalasia than in those with non-specific motor disorders. MR-fluoroscopy approach represents a promising radiation-free modality in the evaluation of esophageal motor activity assessing wall contractility, motor coordination, and transit.

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Microstructural volumetric imaging of a human gastroesophageal junction

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Aim: To combine the micro-anatomy (muscle distribution and fiber orientation) and physiology of the GEJ into a 3D computational model to better understand the relationship between the anatomy and physiology of the GEJ. **Background:** A realistic 3D anatomically-based computer model of the GEJ has been constructed using cross-sectional images from the Visible Human Project. The boundaries of the esophagus were manually traced on each slice (260 slices, 2 mm apart) and used to construct the 3D computer model with accurate geometric information. We seek to augment the geometrical model with microstructural details on the fiber orientation and distribution using a large volume imaging system. **Methods and results:** An *en-bloc* harvest of the GEJ from a cadaver has been imaged to obtain detailed microstructural information such as muscle fiber size and orientation. The tissue was excised 10 hrs after death and suspended in a specially designed jig to maintain anatomical relations. The sample was fixed in 3% formalin for 1 week before wax embedding resulting in 75x75x55 mm^3 sample block. A custom built extended-volume imaging system comprising a digital camera (Canon1D MarkII) and Ultramill (Leica SP2600) mounted over a precision (0.1μm step) XYZ stage (Aerotech) was used to obtain detailed microstructural information using Surface Imaging Microscopy. The surface of the block is milled flat, etched, stained with May-Grunwald and a 6x6 overlapping matrix of images captured to cover the entire surface at 8.22 μm pixel resolution. This cycle is then repeated at 50 μm Z steps. The stained tissue is segmented out from the image which is effectively a 3 μm optical section with resolution comparable to conventional histological thin sections. The fiber information will be extracted and used to augment the 3D model. The model will be used to simulate a normal swallow by solving the laws of mechanics that govern finite deformation elasticity and results will be compared to experimental recordings. **Conclusions:** The model is a framework which can be used to examine the roles of micro-anatomy in the functionality of the GEJ. The model will provide opportunities to examine the relationship between anatomy and physiology in health and disease and allow the determination of the contributions of different elements of anti-reflux surgery.

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Adenosine modulates esophageal sensorimotor function in healthy humans

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Introduction/Aim: The neurohumoral mechanisms responsible for esophageal hypersensitivity are unclear. Adenosine stimulates vagal nociceptors and has been implicated as a mediator for somatic and visceral pain. However, the effects of adenosine on sensory and biomechanical properties of the esophagus are unknown. Our aim was to investigate the effects of adenosine on esophageal sensorimotor function. **Methods:** In a double blind, randomized, placebo-controlled study, 14 healthy volunteers (M/F= 4/10, mean age 37 years) received either adenosine or placebo infusion intravenously at a rate of 100 μg/kg/min. During infusion, all subjects underwent stepwise graded balloon distensions of the esophagus using impedance planimetry. Sensory responses and biomechanical properties were assessed. Distensions were discontinued when subjects reported pain or a distension pressure of 65 cm H_2O was reached. Sensory thresholds were evaluated using a 4 point Likert scale. One hour before the infusion, a baseline impedance planimetry was performed to assess basal sensorimotor properties of the esophagus. Data were analyzed using ANOVA and paired t test. **Results:** After adenosine, the cross sectional area (CSA) of the esophageal wall increased (p<0.05*) and the circumferential wall tension/strain relationship shifted to the left (wall became stiffer) (p<0.05*), when compared to baseline or placebo. Adenosine also significantly lowered sensory thresholds for first perception (mean ± SD, cm H_20; 14 ± 5 vs 25 ± 5, p< 0.004), discomfort (38 ± 6 vs 52 ± 13, p< 0.04) and pain (51 ± 8 vs 65 ± 7, p< 0.04) when compared to placebo. **Conclusions:** Adenosine lowers esophageal sensory thresholds and decreases esophageal distensibility. These sensorimotor changes of the esophageal wall are identical to those reported consistently in patients with functional esophageal chest pain and hypersensitivity. Thus, adenosine could serve as a key neuromediator in the pathogenesis of functional chest pain and esophageal hyperalgesia.

Balloon pressure →	10 cm H_20			40 cm H_20		
	Baseline	Adenosine	Placebo	Baseline	Adenosine	Placebo
CSA ($mm^{2)}$	120 ± 28	178 ± 27*	121 ± 15	399 ± 77	558 ± 94*	358 ± 58
Tension (mm^2 / cm H_20)	61± 6	77 ± 7*	62 ± 5	448 ± 45	530 ± 67*	426 ± 37
Strain	0.21± .1	0.15 ± .04*	0.26 ± .1	1.2 ± .15	0.99 ± 0.1	1.19 ± .1

* p < 0.05

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Esophageal barostat or impedance planimetry: which is best suited for esophageal sensory testing?

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Introduction/Aim: Balloon distension of the esophagus using either a barostat or impedance planimetry is commonly used for the assessment of sensory properties. Although useful, the pros and cons of each technique is unknown. Our aim was to assess and compare the utility and tolerability of balloon distensions using an esophageal barostat (EB) or impedance planimetry (IP) in healthy humans. **Methods:** Sixteen healthy volunteers (M/F= 6/10, mean age 37 years) underwent stepwise graded balloon distensions of the esophagus using either IP (n=8) or EB (n=8). EB was performed by placing a 5 cm long, highly compliant balloon, and IP by placing a 5 cm long latex balloon, and both were located 10 cm above LES. With both techniques, intermittent phasic distensions were performed at increments of 6 mm Hg until the subject reported pain or a maximum distension pressure of 48 mmHg was reached. Sensory thresholds were evaluated using a 4 point Likert scale. Biomechanical properties were also assessed. The success rate and tolerance of each procedure was also evaluated. **Results:** Sensory thresholds for first perception (mm Hg, mean ± SD; 21 ± 6 vs. 21.2 ± 5, p=0.9), discomfort (38 ± 8 vs. 35 ± 9, p=0.5), and pain (44 ± 4 vs. 45 ± 3, p=0.7) were similar between IP and EB. However, only 4/8 (50%) subjects tolerated barostat balloon distensions when compared to 7/8 (88%) subjects for IP induced balloon distensions (p<0.05). The reasons for intolerance with the EB included, excessive forceful pulling of the distended balloon due to strong esophageal peristalsis (n=4), pulling around the angle of the mouth (n=4), chest discomfort (n=2) and severe retching (n=2). In 2 subjects the balloon was propelled into the stomach. Throat discomfort was reported by 4/8 with EB and 3/8 with IP. **Conclusions:** Impedance planimetry provides equivalent information regarding esophageal sensory properties, but is significantly better tolerated than esophageal barostat. The inability to tolerate EB may be related to the size of the balloon, rate of inflation or the use of fixed balloon distending pressure as opposed to a more conformable yet dynamic balloon distending system with IP. Hence, although barostat is more widely available and suitable for gastric, colonic and rectal functional assessments, at present, IP appears to be superior to EB for routine assessment of esophageal

sensory testing. Additionally, IP allows evaluation of esophageal biomechanical properties such as cross sectional area and tension/strain relationship, and peristaltic properties.

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Endoscopic pneumatic dilation in Chagasic Schalasia: A clinical, radiological and manometric study with one year follow-up

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Pneumatic dilation (PD) of the cardia is a widely accepted treatment of achalasia. Pneumatic balloon dilation under endoscopic vision has been recently used. There are few prospective, longitudinal studies evaluating clinical, radiological and manometric outcomes after endoscopic dilation in patients with chagasic megaesophagus. **Aim:** To evaluate symptom improvement, radiological and manometric alterations after pneumatic dilation under endoscopic vision in patients with chagasic achalasia, one month and one year after PD. **Patients & Methods:** Thirty two patients with Chagasic megaesophagus (17 men, mean age 55) were studied. All patients had clinical, radiological and manometric examinations before, one month and one year after PD. Body weight and symptom score (0–3) for dysphagia, regurgitation, chest pain and heartburn were accessed in all patients. The esophageal diameter and barium height (BH) 1 min after ingestion were measured. Basal end-expiratory lower esophageal sphincter pressure (LESP) was evaluated before, one-month and one year after PD. PD was performed using a pneumatic balloon dilator (Montag, Brazil) with 3,5mm diameter, under pressure of 7–8 psi, for 1 min, under direct endoscopic vision. **Results:** There was clinical improvement (less dysphagia, regurgitation and chest pain; weight gain - 5kg) one month that remained after one year. LESP decreased 30% (6,34 mmHg) one month after PD (p<0,001). LESP pressure one year after PD was similar to that of 1 month after PD(p=0,43). Esophageal diameter decreased 10% after one-month and 36% after one year. Barium height column (BH) decreased 59% one-year after PD. There was one esophageal perforation (1/32; 3,5%) surgically treated. **Conclusions:** Pneumatic dilation of the cardia under direct endoscopic vision, in patients with Chagasic achalasia, using Montag (Brazil) balloon, result in clinical improvement, decreased LESP pressure and esophageal diameter that remain for one year after PD.

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Modulation of vagally induced twitch contractions by a local reflex circuit involving primary sensory afferents and enteric neurons in the mouse esophagus

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Motor endplates in the striated muscle of the mammalian esophagus receive a dual innervation from both vagal nerve fibers originating in the brain stem and from varicose enteric nerve fibers originating in the myenteric plexus. Enteric neurons in the esophagus are contacted by primary afferents of spinal and vagal origin. A substantial number of these primary afferents, mainly of spinal origin, were shown to be immunoreactive to the transient receptor potential ion channel of the vanilloid type 1 (TRPV1) and called capsaicin-sensitive sensory neurons. The local release of peptide mediators, such as substance P (SP) and calcitonin gene-related peptide (CGRP), from peripheral sensory endings enables these neurons to exert a local effector action. The aim of the present study was to investigate the components of a local reflex circuit that is proposed to influence the vagally induced contractions of the mouse esophagus using capsaicin. For this purpose, a thoracic esophageal segment including vagus nerves was dissected from adult ddY mice and placed in an organ bath. Because contraction amplitude of peristalsis is mainly a function of esophageal circular muscle contraction, we measured the contractile activity in the circular direction with a force transducer. Vagal stimulation (30μs, 25V, 1–50Hz for 1sec) produced monophasic contractile responses, whose amplitudes were frequency-dependent. These contractions were significantly inhibited by d-tubocurarine while resistant to atropine and hexamethonium, indicating that the vagally evoked twitch contractile responses are mediated by acetylcholine (ACh) via its neuromuscular nicotinic receptor subtype located on the motor endplates. Capsaicin inhibited the vagally evoked contractions in a dose-dependent manner. SB-366791, a novel selective TRPV1 antagonist, blocked the inhibitory effect of capsaicin. Additionally, the inhibitory effect of capsaicin was blocked by L-732,138 and L-NAME, antagonists of NK1 receptor and nitric oxide synthase respectively. Taken together, we conclude that the activation of capsaicin-sensitive sensory afferents can suppress the vagally mediated striated muscle contraction in mouse esophagus through a local reflex circuit involving nitrergic myenteric neurons.
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Proton pump inhibitor (PPI) influence on reflux clearance in Barrett's oesophagus

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In healthy controls (HC) acid reflux is cleared by increased peristaltic wave activity, but this mechanism appears flawed in Barrett's oesophagus (BO) patients. If duodeno-gastro–oesophageal reflux (DGOR) is cleared by the same mechanism, gastric acid suppression may affect its removal from the oesophagus. Studies comparing motility during reflux in these two groups both with and without PPI therapy may elucidate this removal mechanism.

18 HC (11males) mean age 26(19–45) years and 12 BO patients (7 males) mean age 58(42–69) years were investigated. All BO patients were on PPI therapy (omeprazole 40mg od). pH, % peristalsis, and wave activity (waves per minute) were measured using a 3-pressure transducer catheter and antimony pH tip (Konigsberg, USA), connected to a Flexilog 3000 datalogger, and data was analysed using Flexisoft software (Oakfield Instruments Ltd, UK). DGOR was measured using a sodium ion selective electrode (Microelectrodes Inc.,USA), and Axum graphical analysis software (Adept Scientific, UK) was used for calculation. The catheter was placed 5 cm above the lower oesophageal sphincter and upright, supine and meal time periods were noted. All subjects were studied on two occasions (healthy controls also after 20mg of omeprazole bd for 2 days, and patients also after 5 days without medication).

Without PPI therapy, more upright acid reflux (pH < 4) occurred in BO patients, 26%, compared to HC, 2.9% p=0.009. (Mann-Whitney U test)). Increased upright DGOR occurred in BO, 61% compared to HC, 22%. There was no significant difference in wave activity, BO 1.4 , HC 1.7, but more % peristalsis occurred in HC, 64% than BO, 53% , p=0.01, during upright acid reflux.

With PPI therapy, upright acid reflux was still higher in BO group, 9.9%, compared to HC, 1.6% , p=0.009. There was no significant difference in DGOR between the 2 groups, BO 31% , HC 19 %. More distal wave activity occurred in HC, (3.8) compared to BO (1.4) . There was no difference in % peristaltic waves between the 2 groups, HC= 67%,BO= 56%, during upright acid reflux.

Without acid suppression, patients with Barrett's oesophagus produce fewer co-ordinated waves to clear the oesophagus compared to healthy controls. The addition of a PPI reduces DGOR in the Barrett's group, the reduction possibly related to improved wave co-ordination.

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G protein β3 subunit 825 T allele is associated with disease susceptibility and visceral hypersensitivity in GERD

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Background: A genetic predisposition to GERD has been demonstrated by familial clustering and twin studies. Furthermore, the role of

visceral hypersensitivity in the pathophysiology of GERD symptom generation is increasingly recognized. Putative genetic factors may be related to visceral hypersensitivity. Visceral hypersensitivity may arise from lowered sensory thresholds of esophageal afferent terminals. The enhanced production of IL-8 in the esophageal mucosa of even patients with endoscopically negative reflux disease, implies that inflammatory substances are increased in GERD. These can activate protease-activated, bradykinin, and prostaglandin receptors, all G protein coupled receptors (GPCRs), on esophageal afferent terminals. Moreover, this may lead to heterologous sensitization of vanilloid receptor 1, an acid-sensing transducer protein, on esophageal afferent terminals. Besides, increased responsiveness of GPCRs involved in the processing of visceral stimuli in the spinal cord (receptors for substance P and calcitonin gene-related peptide) may contribute to visceral hypersensitivity. Therefore, it is conceivable that G proteins mediating enhanced signal transduction may increase visceral sensitivity. The C825T polymorphism of the GNB3 gene, coding for the $\beta 3$ subunit of heterotrimeric G proteins, results in enhanced activity. **Aim:** Assess association of C825T polymorphism in GNB3 with disease susceptibility and phenotype in patients with GERD. **Materials and Methods:** A total of 305 unrelated patients with GERD and 344 healthy controls (HC) were studied. GERD was defined using 24-hour esophageal pH-monitoring and symptom association scores (total time pH<4 ≥6% and/or SI≥50% and/or SAP≥95%). HC were selected using their response to questionnaires to make sure gastrointestinal symptoms were absent. All participants were of Caucasian descent. DNA was extracted from whole blood. Genotyping was performed by molecular beacon assay. **Results:** Carriers of the T allele were more prevalent in GERD patients relative to HC (OR 1.43, 95%CI 1.05–1.95, P=0.022). The patient subgroup (n=56) with physiological acid exposure (total time pH <4 <6%, SI≥50% and/or SAP≥95%), generally considered to be visceral hypersensitive, showed an even higher frequency of the T allele (OR 1.87, 95%CI 1.05–3.35, P=0.032). **Conclusion:** The association identified suggests that GNB3 is a genetic determinant of visceral hypersensitivity as well as susceptibility to GERD.

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Relationship of Nutcracker esophagus with gastroesophageal reflux disease. A pathophysiological approach

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Esophageal manometry is a diagnostic tool indicated in gastroesophageal reflux prior to a surgical treatment to exclude a primary motor disease as achalasia or spastic problems in the body. Our aim is to report the experience with patients with Nutcracker esophagus found in patients refered for evaluation to surgical treatment for reflux disease.

In a period from July 2004 and April 2006, 300 patients were referred for esophageal manometry with the diagnosis or gastroesophageal reflux. All of them had a prior endoscopy with esophagitis. 20 patients had a Nutcracker esophagus. All patients had symptoms of heartburn and regurgitation, 6 patients had chest pain, 4 had dysphagia and only one refered chest pain and dysphagia. A 24 hour pHmetry was performed and patients were integrated into three groups: In group I, with 10 patients, with positive for reflux disease and a medical treatment with a double dose of PPI were installed. In group II, with 4 patients, the pHmetry was negative but with a positive symptom index and a treatment with PPI and imipramine were given. In group III, with 6 patients, the pHmetry was negative and a esophageal dilation was performed.

The patients in group I had a good response in 8, a fair response in the other 2. The patients in group II had a good response after 2 months (3–8 months) and patients in group III had a fair response and after 3 dilations (1–4) only two required treatment with PPI after 3 months treatment (4–10 months)

We concluded that Nutcracker esophagus can be classified into a primary motor disorder as we usually know this entity, but also as a inflammatory response of GERD and a small fraction of patients will have hypersensitivity disease due probably to reflux. However, this is a small sample and a following study will be needed.

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Distension during gastro-esophageal reflux: effects of acid inhibition and correlation with symptom

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Objectives: To determine the effects of acid inhibition and symptom correlation with reflux associated esophageal distension. **Design:** GER was studied using combined multiple intraluminal impedance, esophageal pH, manometry and ultrasound imaging during a 2 hours post-prandial period. **Setting:** Outpatient. **Patients:** 10 controls and 10 GER disease patients (endoscopic or pH proven) off medications for at least 7 days. **Intervention:** GERD subjects were studied before and during esomeprazole 40 mg daily therapy. **Outcome measures:** US images were analyzed to determine esophageal cross sectional area (CSA) during GER. Comparison were made between: 1) controls and GERD patients, 2) symptomatic and asymptomatic GER episodes and 3) before and during esomeprazole therapy in GERD patients. **Results:** Median lumen CSA at peak GER induced esophageal distension is greater in patients than controls (285 vs. 197 mm2, p=0.002). 28 heartburn and 8 chest pain episodes in 10 patients showed differences in lumen CSA between asymptomatic GER episodes and the ones associated with chest pain (411 vs. 266 mm2, p=0.009) but not with heartburn (256 mm2, p>0.05). Following esomeprazole treatment, the majority of GER episodes were neutral and patients asymptomatic except for 2 patients who showed cyclical reflux and reported regurgitation (4), heartburn (10) and chest pain (2). Lumen CSA was not different between the 2 study days. **Conclusions:** Esophageal distension is greater in GERD subjects as compared to controls and for episodes associated with chest pain but not with heartburn. Acid suppression does not alter GER induced esophageal distension. This study was funded by NRSA 5 T32 DK 07202-2 (NT) and by a research grant from Astra-Zeneca (RK).

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Distension of the esophagogastric junction augments triggering of TLESR in healthy volunteers

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Background: The vago-vagal transient lower esophageal sphincter relaxation (TLESR) reflex is the major mechanism of gastroesophageal reflux (GER) triggering initiated by stimulation of gastric mechanoreceptors. Placement of nasogastric tubes of increasing size across the esophagogastric junction (EGJ) increases GER, however the mechanisms responsible for this effect have not been determined. We hypothesized that distension of the EGJ exacerbates triggering of GER via a tension-receptor-mediated mechanism which augments triggering of the TLESR reflex. **Methods:** Ten healthy volunteers (6M:4F, 21–37yrs) were studied using a purpose-built esophageal sleeve-catheter (OD 4.0mm) incorporating an 8cm internal balloon adjacent to the sleeve. This configuration allowed continuous measurement of LES pressure and identification of TLESR during distension of the EGJ to 4.0x10.0mm (oval). The catheter was passed into the esophagus with the sleeve straddling the LES. After 30 minutes of adaptation, the balloon was either inflated or left deflated. After 45 minutes of baseline recording, the subject was given 600 ml of carbonated soft drink (coca cola). Sixty minutes later the balloon was either deflated or inflated, followed by another 45 min of baseline recording, another test drink and 60 min post-drink period. During the entire study period volunteers were asked to report belches. The protocol was repeated on a separate day with the order of distension reversed. Blinded analysis was performed with TLESRs identified using standard criteria. **Results:** Inflation of the balloon was never perceived by volunteers. The number of TLESRs and belches was greater during EGJ distension (table) than without. **Conclusion:** Localized EGJ distension augments triggering of TLESRs and belching suggesting that EGJ vagal tension receptors may play a role in the afferent arm of the TLESR reflex. These data indicate a possible mechanism that may underlie

increased GER triggering in association with the increased EGJ distensibility previously characterized in patients with GERD and/or hiatus hernia.

	Pre drink period			Post drink period		
	Undistended	Distended	p-value	Undistended	Distended	p-value
No. TLESRs	3.0(2.0–4.0)	4.0(4.0–5.0)	0.060	8.5±3.2	9.8±2.5	0.039
No. belches	2.0(0.5–2.0)	3.5(1.0–4.5)	0.010	7.8±3.9	10.3±4.0	<0.001

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Do pelvic ganglia functionally innervate the aganglionic rectum of piebald-lethal mice?

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Piebald-lethal mice (s^l/s^l) fail to express the endothelin B receptor (*EDNRB*), resulting in rectal aganglionosis and megacolon. In unaffected animals extrinsic pelvic efferents innervate myenteric ganglia, but in aganglionic regions (ie. s^l/s^l mice) it is not known if the pelvic efferents play a functional role in motility. We investigated whether stimulation of pelvic ganglia in s^l/s^l mice would modify colonic function. Mice were anesthetized with isoflurane and killed by cervical dislocation. The whole colon with one attached pelvic ganglion was removed and placed in a divided organ bath where either 1) the distal portion of the colon was opened along the mesentery and pinned serosa up for intracellular recordings from the circular smooth muscle (CM), or 2) the colon was attached to tension transducers in the proximal, mid, and distal areas for recording the migrating myoelectric complex (MMC). Pelvic ganglia were stimulated with DMPP (100μM), 5-HT (500nM) or ATP (100μM). Data shown as mean±SEM. In C57 mice, spontaneous and electrically evoked IJPs were always detected (mean amplitude, 14±2mV, n=7). Intracellular recordings from CM cells in the aganglionic rectum (n=9) revealed an absence of spontaneous activity, and no response to enteric electrical stimulation (1–5 pulses, 5mA, 1ms). Stimulation of pelvic ganglia in C57 mice (n=7) resulted in hyperpolarization of the CM of 14±2mV (n= 4-ATP, 5–5-HT, 6-DMPP). DMPP, 5-HT or ATP applied to pelvic ganglia in s^l/s^l mice had no effect on aganglionic CM membrane potential, despite a large hyperpolarization induced by direct application of ATP to the muscle (1mM, 14±3mV, n=8). Contractile recordings from C57 mice (n=5) showed coordinated MMCs along the entire colon. These were absent in s^l/s^l mice (n=5). In C57 mice, pelvic stimulation abolished MMCs along the entire colon. However, in s^l/s^l mice, stimulation of pelvic ganglia had no effect on the mechanical activity of the aganglionic rectum, but surprisingly relaxed the CM in mid and proximal colon. Labeling of single pelvic nerve trunks in C57 mice revealed that afferent and efferent nerve endings ramified almost exclusively in myenteric ganglia. However, in the aganglionic s^l/s^l mice, pelvic nerves usually terminated in intramuscular arrays (n=5). Quantitative PCR studies revealed the presence of P2Y1, M2, M3, B2 and B3 receptors in the aganglionic rectum. In summary, activation of the pelvic ganglia in s^l/s^l mice appears to have no direct functional control over the aganglionic rectum but surprisingly is able to relax the musculature in more proximal regions of colon, where enteric ganglia are present.

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Luminal stimuli acutely sensitize viscermotor responses to distension of the rat stomach

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We have previously shown that inflammation enhances responses to gastric distension and intragastric instillation of chemical stimuli consistent with the development of visceral hypersensitivity. As most visceral afferents are polymodal, we hypothesized that sequentially applied innocuous stimuli interact and acutely sensitize responses. **Methods:** Visceromotor responses (VMR) to gastric stimuli were measured in male Sprague Dawley rats by recording electromyographic (EMG) activity from the acromiotrapezius muscle. Mechanical stimulation was performed with a chronically implanted balloon, delivering stepwise distension from 10 – 80 mmHg. Saline, glycocholic acid (GCA) or ethanol were administered through a gastrostomy catheter. Gastric distension was performed at baseline and 60 min after instillation of the second stimulus in healthy animals and rats with gastric ulcers. In a second series of experiments, acid was administered before and 60 min after repeated noxious distension of the stomach. **Results:** Stepwise gastric distension triggered increasing VMR at pressures exceeding 30 mmHg. Instillation of 50 % ethanol, but not saline or GCA increased acromiotrapezius EMG activity in controls with a more significant rise in animals with gastric ulcerations. Only GCA increased responses to gastric distension in control rats, while both GCA and ethanol enhanced responses to gastric distensions in rats with gastric ulcers. Responses to intragastric saline, bile acid or ethanoal were not affected by repeated noxious distension of the stomach. **Conclusion:** Luminal stimuli can trigger visceromotor responses and sensitize gastric afferents to mechanical stimulation. This acute effect may contribute to dyspeptic symptoms triggered by food intake.

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Dichotomizing axons in spinal and vagal innervation of the mouse stomach

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Visceral sensory input is typically poorly localized, which may in part be due to branching of peripheral terminals with more than one receptive field within the target organ. We hypothesized that gastric sensory neurons frequently dichotomize innervating more than one anatomically distinct region.

Methods: The neurochemical phenotype and projections of gastroduodenal sensory neurons were determined in adult male C57BL6 mice. Choleratoxin B (CTB) coupled to different fluorophors was injected along the greater curvature into fundus, corpus, antrum and/or distal duodenum. Immunoreactivity for TRPV1, neurofilament (N52), calcitonin gene related peptide (CGRP) and presence of isolectin B4 (IB4) as well as double labeling for different retrograde labels was determined. **Result:** CTB-injection into fundus and pylorus did not result in any overlap of tracer within the stomach. However, 36±11 % and 20±7 % of dorsal root ganglion (DRG) and nodose ganglion (NG) neurons showed double labeling. Tracer injections into fundus and distal duodenum caused double labeling in 8±5 % and 8.0±7 % of DRG and NG neurons, respectively. Most DRG neurons showed immunoreactivity for TRPV1 and CGRP (TRPV1: 77±2 % and 59±3 %; CGRP: 83±6 % and 82±5 %; values for stomach and duodenum). In contrast, about half of the gastric NG neurons had TRPV1 immunoreactivity (51±9 %) or showed IB4 labeling (41±3%) with only 10 % CGRP-positive neurons. N52 immunoreactivity was present in one fourth or gastroduodenal DRG and NG neurons. **Conclusion:** Retrograde labeling from anatomically distinct areas suggests that a substantial number of DRG and NG neurons innervate both stomach and duodenum. Neurons with such dichotomizing axons may contribute to the poor ability to localize or discriminate visceral stimuli.

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Metabotropic glutamate 5 receptors contribute to colorectal mechanical sensitivity in rats

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Background: The metabotropic glutamate 5 receptor (mGluR5) has been suggested to be involved in nociception. 2-methyl-6-(phenylethynyl)- pyridine (MPEP), a mGluR5 receptor antagonist, is effective in animal models of inflammatory and neuropathic pain. The effects of MPEP on visceral pain pathways from the gastrointestinal tract have

not been characterized. We evaluated effects of MPEP on mechanosensory responses of distal colonic splanchnic afferents in vitro, plus the colorectal distension (CRD)-evoked visceromotor response (VMR) in conscious rats. **Methods:** Distal colons were removed from Sprague-Dawley rats with attached lumbar splanchnic nerves and pinned flat in a specialized organ bath for single afferent fibre recording. Mechanosensitive serosal afferent endings were identified by blunt probing. Conscious Sprague-Dawley rats (n=8 for each dose) were subjected to repeated, isobaric CRD using a paradigm of 12x80mmHg, for 30s with 5min intervals. The phasic changes in intracolonic balloon pressure during the distension period, which reflect abdominal contractions (i.e. VMR) were monitored. MPEP (1–10μmol/kg) or saline was administered intravenously between distensions 3 and 4. **Results:** Serosal probing elicited reproducible bursts of action potentials in splanchnic afferents (8.4±0.3 AP/0.5sec). This response was reduced to 3.7±1.1 AP/0.5sec by MPEP 30 μM (P<0.01, n=5), which was shown in separate experiments to be dose-dependent over the range 1–30 μM. In conscious rats, CRD at 80mmHg resulted in a significant VMR which increased approximately 2-fold during the repeated distension paradigm in saline-treated animals. MPEP reduced the VMR to CRD in a dose-dependent manner. At doses of 6 and 10 μmol/kg, MPEP inhibited the response to CRD by 54.1±13.2% (P < 0.05) and 76.6±7.3% (P<0.01), respectively. Lower doses (1 and 3 μmol/kg) had no significant effect. MPEP (10 μmol/kg) did not affect pressure-volume relationships in the colon. No gross effects on motor coordination were seen at any of the doses tested. **Conclusions:** The results demonstrate that MPEP inhibits CRD-evoked VMR in conscious rats, and this may in part occur at the level of the peripheral afferent ending. These observations suggest that mGluR5 receptors participate in mediating mechanically evoked visceral nociception in the gastrointestinal tract.

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Activation of the TRPA1 ion channel induces mechanical hypersensitivity of colonic splanchnic afferents

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Background: Hypersensitivity of the colon to mechanical stimuli is a characteristic feature of IBS. Mechanotransduction mechanisms of colonic afferents are therefore important targets. We have shown that the ion channels ASIC1, 2, 3 and TRPV4 contribute to these processes. TRPA1 is novel candidate mechanotransduction channel recently implicated in nociception[1]; it is unknown whether it contributes to colonic mechanosensation. **Aims:** 1) To compare the relative expression of TRPA1 transcripts in dorsal root ganglia (DRG) with expression in identified colonic sensory neurons within these ganglia. 2) To investigate the role of TRPA1 in colonic afferent mechanotransduction. **Methods:** Quantitative RT-PCR was used to determine TRPA1 mRNA expression in whole thoracolumbar (T10-L1) and lumbosacral (L6-S1) DRG, which contain the cell bodies of colonic splanchnic afferent neurons. QRT-PCR was also used to determine expression specifically in colonic DRG cells identified by retrograde labelling with CTB-FITC that were isolated via laser capture microdissection. An *in vitro* preparation of mouse colon[3] with attached splanchnic nerves was used to examine the mechanosensitivity of serosal and mesenteric afferents to graded probing forces before and after the addition of the TRPA1 agonists allyl-isothiocyanate (40μM)[4] and Trans-cinnamaldehyde (100μM)[4]. **Results:** QRT-PCR revealed TRPA1 mRNA expression in whole TL DRG in greater abundance than ASIC1, ASIC3 or TRPV4 mRNA. In laser-captured colonic DRG cells, QRT-PCR revealed a high level of expression of TRPA1. The TRPA1 agonist allyl isothiocyanate (40μM) caused a significant and potent increase in the mechanical stimulus response functions of splanchnic colonic mesenteric (P<0.01, 2-way ANOVA, n=8) and serosal (P<0.01, n=18) afferents. Trans-cinnamaldehyde (100μM) similarly caused a similar significant and potent increase in the responsiveness of both classes of afferents to mechanical stimuli. **Conclusions:** TRPA1 is expressed in colonic neurons and activation of TRPA1 evokes mechanical hypersensitivity of colonic afferents. TRPA1 may therefore contribute to the mechanical hypersensitivity that leads to pain in IBS. Corey et al., 2004, Nature 432:723-730. Kwan et al., 2006, Neuron 50, 277–289. Brierley et al., Gastroenterology 127:166–178. [4] Bandell et al., 2004. Neuron 41:849–857. Supported by NHMRC Australia.

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Experimental colitis in rats impairs gastric emptying via the pelvic nerve

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Background: Patients with inflammatory bowel disease often suffer from motility disturbances at sites remote from the inflammation. We previously demonstrated a neuronally mediated delay in gastric emptying (GE) in rats with experimental colitis probably mediated via TRPV1 receptor activation, in the absence of gastric inflammation[1]. Our current **aim** was to further investigate the involvement of extrinsic neuronal pathways within the pelvic nerve, which innervates the bladder and the distal colon. **Methods:** Distal colitis was induced in male Wistar rats by a colorectal enema of 7.5 mg trinitrobenzene sulphate (TNBS) in 50% ethanol 72 h prior to experiment. GE was measured 30 min after intragastric instillation of a semi-liquid bolus of Evans blue. Rats underwent laparotomy 7d prior to experiments. The pelvic nerve was isolated near the iliac bifurcation and cut. In sham-operated rats, the nerve was only isolated. After surgery, the bladder was manually drained twice daily. The effect of nerve section on the inflammatory response was assessed using a myeloperoxidase (MPO) assay. To confirm the role of pelvic nerve afferent neurons in colitis-induced gastroparesis, we stained the dorsal root ganglion (DRG) S1 for the expression of c-fos in TNBS-treated rats and controls, with or without prior colonic distension (60 mmHg, 45 min). **Results:** TNBS-colitis significantly reduced GE in sham-operated rats from 38.4±4.6 % to 19.8±5.3 % (P<0.05, n=8–12). Pelvic nerve section had no significant effect on GE in control rats (43.5±5.5 %) but normalised it in TNBS-treated rats (52.5±6.3 %). This procedure had no effect on the MPO content of the colon wall which was 30.3±9.5 U/g in sham-operated TNBS-rats and 36.4±4.8 U/g in TNBS-rats with pelvic nerve section (NS, n=5). In the absence of prior colonic distension, no c-fos immunoreactivity was identified in DRG S1 of control rats whereas 0.29±0.03 % of cell bodies stained positive in TNBS-treated rats (P<0.05, n=2). After colonic distension, 0.33±0.01 % of neurons expressed c-fos in control rats compared to 0.58±0.01 % in TNBS-treated rats (P<0.05, n=2). **Conclusion:** Experimental colitis in rats impairs gastric emptying via a neuronal reflex pathway which involves primary afferent neurons within the pelvic nerve. Our c-fos experiments suggest an inflammation-induced recruitment phenomenon is responsible for these effects. [1]De Schepper et al. Effect of hexamethonium and different TRPV1 receptor antagonists on impaired gastric emptying induced by experimental colitis in rats. Gastroenterology 2006;130:A258.

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Role of afferent neurons and TRPV-1 receptors in the pathogenesis of septic ileus in mice

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Aim: Sepsis is often associated with gastrointestinal motility disturbances. In this study, we investigated the role of extrinsic afferent neurons and TRPV-1 receptors in septic ileus in mice. **Methods:** Septic ileus was induced by an ip injection of lipopolysaccharides (LPS,

E. coli, 20 mg/kg). Mice received an intragastric injection of 0.1 ml Evans blue 18 h after LPS. Gastric emptying (GE) was measured spectrophotometrically and small intestinal transit was measured as the migration of Evans blue, 15 min after injection of Evans blue. In the first series of experiments, mice were treated with a neurotoxic dose of capsaicin (125 mg/kg, sc) or its solvent (Solvent 1: 10% Tween 80–10% ethanol-80% saline) 14 days prior to the induction of sepsis. In the second series of experiments, mice were treated 1 h before administration of Evans blue with the TRPV-1 antagonist IRTX (5'-iodo-resinferatoxin, 75 μg/kg, ip) or its solvent (Solvent 2: 10% DMSO) or with the high potency selective TRPV-1 antagonist BCTC (*N*-(4-tertiary butylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2*H*)-carbox-amide, 20 mg/kg, ip) or its solvent (Solvent 3: 25% hydroxypropyl-β-cyclodextrine) in the third series of experiments. **Results:** (Table): Injection of LPS resulted in a significant delay in GE and intestinal transit in solvent-treated mice. Capsaicin and BCTC both had no effect on GE or intestinal transit in control mice but completely reversed the LPS-induced delay in GE and significantly reduced the LPS-induced delay in intestinal transit. IRTX had no significant effect on GE or transit in control or septic mice. **Conclusion:** These results suggest a role for capsaicin-sensitive afferent neurons most likely mediated via TRPV-1 receptors in the pathogenesis of septic ileus in mice. IRTX did not show the beneficial effects probably due to poor pharmacokinetic properties in vivo.

		Solvent 1	Capsaicin	Solvent 2	IRTX	Solvent 3	BCTC
GE	Control	64.4±5.2	54.2±7.2	41.1±5.5	51.7±5.2	57.0±7.3	45.6±8.1
	LPS	36.3±2.7*	59.2±7.7#	25.7±4.9*	22.5±6.1*	19.7±5.8*	56.6±6.0#
Transit	Control	42.3±3.6	37.5±3.6	49.2±3.0	47.3±2.2	47.6±3.8	47.4±3.5
	LPS	20.9±1.3 *	29.1±3.5#	30.4±2.7*	23.6±5.6*	18.9±4.7*	31.2±3.5*#

*P≤0.05 compared to control mice treated with the same solvent or the same drug; # P≤0.05 compared to solvent-treated LPS mice (two-way ANOVA, n=9–11 in each group).

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Behavioural disruption of human swallowing reaction times following a 1Hz repetitive transcranial magnetic stimulation induced virtual lesion of swallowing motor cortex

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Background: 1Hz repetitive transcranial magnetic stimulation (rTMS) can be used to inhibit cortex to induce virtual lesions of targeted brain regions. We applied a 1Hz virtual lesion to healthy human swallowing motor cortex to establish whether such inhibition might induce behavioural changes to swallowing. **Methods:** 8 healthy subjects (3F; range 26–47 years) were recruited. Single pulse TMS (sTMS) was applied to swallowing motor cortex (SMC) to elicit pharyngeal motor evoked potentials (PMEPs) recorded via a swallowed intraluminal catheter. The hemisphere evoking the largest PMEPs was termed the dominant SMC (DSMC). Following baseline sTMS recordings of both ipsilateral DSMC and contralateral non-dominant SMC (NDSMC), a baseline swallowing reaction time task was performed comprising 3 cued tasks: normal, fast and challenged swallows (within a 150ms time window). Thereafter, 10 min of rTMS (real or sham, randomised to separate days) was applied to the DSMC. sTMS was then reapplied, immediately, 30 and 60 min after rTMS (or sham), in conjunction with the swallowing tasks. Baseline vs. intervention data (real and sham) were then analysed with ANOVA. **Results:** PMEP amplitudes from the DSMC decreased by 21%, after real but not sham rTMS, p<0.01, returning to baseline by 60 min (Figure 1a). After real rTMS, swallow reaction times were reduced by 11% for both normal and fast swallows compared to baseline, p<0.001 not seen with sham rTMS (Figure 1b). Challenged swallows failed to improve with real rTMS, but showed an expected rise in successful hits with sham (p<0.05). **Conclusion:** Our data show a clear relationship between the SMC, excitability and swallowing performance and provides a human model for cortical dysfunction after a virtual brain lesion.

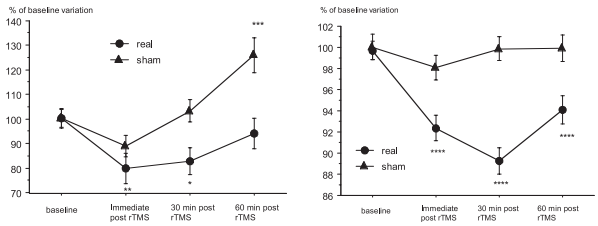

Figure 1 a) Change in PMEP amplitude after real and sham rTMS. (***p<0.001; **p<0.01; *p<0.05). b) Change in normal swallow reaction time after real and sham rTMS (**** p<0.0001).

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***Nippostrongylus brasiliensis*-infected mice exhibit chemical but not mechanical hypersensitivity**

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Background: Nb infection results in a transient inflammation and subsequent increases in mucosal innervation, mast cells and mast cell-nerve contacts. Previous studies have reported hypersensitivity to intestinal distension, but chemosensitivity has not been investigated. This study determined if Nb infection had any effect on mechanosensitive and chemosensitive responses in the murine jejunum. **Methods:** Balb/c mice were infected with 500 L3 Nb larvae or saline (sham) and the following three separate studies performed 3–4 weeks later. Aortic blood pressure was recorded in response to phasic (30 s) balloon distension with up to 0.9 ml, which evoked an intraluminal pressure (IP) of 115.9 ± 6.5 mmHg. Mesenteric afferent nerve activity and IP were recorded in response to either ramp balloon distension up to 60 mmHg, or to a 2.5 min intraluminal perfusion (0.15 ml/min) of 50 mM hydrochloric acid (HCl). Data are expressed as mean ± SEM and analysed with 2-way ANOVA or unpaired t-tests. **Results:** Pressor responses were elicited by phasic distension. There was no significant difference in the pressor responses elicited in sham (n = 18) and infected (n = 13) animals (p = 0.87). Ramp distension evoked an increase in afferent nerve activity. However, afferent responses in Nb infected mice (n = 49) were not significantly different (p = 0.94) from sham mice (n = 48). In response to HCl perfusion there was a rapid increase in afferent activity (acute response) that plateaued within 5 min (prolonged response), and a gradual IP increase that peaked within 5 min. There was an increase in both the afferent and IP responses to HCl in infected (n = 26) compared to sham (n = 25) animals as detailed in the table below. There was no significant direct correlation between any changes in nerve activity and IP. **Conclusions:** Nb infection evokes jejunal hypersensitivity to chemical but not mechanical stimuli. It is postulated that the Nb-induced inflammation mainly impacts the

mucosa which alters the sensitivity of chemosensitive afferents, whilst having little impact on deeper mechanosensitive afferents.

Measurement	Nerve (Δ imp/s)		IP (Δ mmHg)	
Response period	Acute	Prolonged	Acute	Prolonged
Sham	41.6 ± 4.9	27.3 ± 3.6	0.09 ± 0.08	0.34 ± 0.06
Inf	53 ± 3.8	40.5 ± 4.2	0.18 ± 0.09	1.28 ± 0.31
p value	0.07	0.02	0.46	0.005

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Extrinsic afferents supplying the murine jejunum express functional TRPA1 receptors

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Background: TRPA1 is expressed in sensory ganglia and has been reported to play a role in the transduction of thermal, mechanical and chemical stimuli. The role of TRPA1 in the gastrointestinal tract is unknown. The aim of this study was to determine if extrinsic afferents supplying the gut express functional TRPA1 receptors and to characterize some of the properties of human TRPA1 receptors. **Methods:** Mesenteric afferent recordings were made using standard extracellular recording techniques from mouse jejunal afferents. HEK 293 cells were stably transfected with human TRPA1 cDNA. Whole cell patch clamp recordings using TRPA1 agonists allyl isothiocyanate (AITC) and benzyl isothiocyanate (BITC), and the putative TRPA1 antagonists ruthenium red (RR), gadolinium (Gd^{3+}), amiloride and gentamicin were performed. IP injection of Cholera toxin B-488 in Balb/c mice identified DRG (T10 to T13) and nodose neurons projecting to the abdominal viscera, which were isolated using laser-capture microdissection and RNA was hybridized to Affymetrix Mouse whole genome arrays. **Results:** AITC evoked a dose-dependent activation of mesenteric afferent nerves, with an increase in afferent firing from 16.3 ± 5.8 to 44.2 ± 10.4 imp/s, indicating that jejunal afferent express functional TRPA1 receptors. In human TRPA1 transfected cells, AITC and BITC evoked dose-dependent inward currents. BITC (3μM) evoked a peak amplitude of −602.2 ± 48.4 pA (n = 23) and activation time of 17.8 ± 1.4 s (n = 23). The inward current evoked in response to either BITC or AITC was sensitive to both extracellular calcium and holding potential. The response to AITC (10μM) was inhibited by RR (50μM) and amiloride (500μM), but not by Gd^{3+} (100 (M), and gentamicin (50μM). In microarray analysis, TRPA1 mRNA was detected at high levels in both nodose and DRG neurons supplying the abdominal viscera, with 7-fold higher expression in the DRG ($q<0.001$). Furthermore, TRPA1 expression was elevated following infection with the intestinal nematode *Nippostrongylus brasiliensis*, but only in nodose neurons (2.1 fold increase). **Conclusions:** These data demonstrate that TRPA1 receptors are expressed on abdominal DRG and nodose neurons, and that they form functional receptors on intestinal afferent terminals. TRAP1 receptors are potently activated by AITC and BITC, and inhibited by RR and amiloride. The observation that TRPA1 is upregulated in nodose neurons following nematode infection suggests that vagal intestinal TRPA1 receptors may be of pathophysiological relevance.

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Major deficits in visceral mechanosensory function in mice lacking the TRPV4 ion channel

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Background: Colonic sensory neurons reside in thoracolumbar (T10-L1) and lumbosacral (L6-S1) dorsal root ganglia (DRG), projecting via splanchnic and pelvic nerves respectively[1]. Several candidate molecules exist as mechanotransducers in colonic afferents, including the ion channel ASIC3[2], but others include TRPV4 - a transient receptor potential ion channel, recently proposed to transduce mechanical stimuli[3]. **Aims:** To compare the localisation and relative expression of TRPV4 transcripts in whole DRG with expression in identified colonic DRG neurons. 2) To investigate the role of TRPV4 in colonic afferent mechanotransduction. **Methods:** Cells expressing TRPV4 mRNA in T10-L1 and L6-S1 DRG were counted after fluorescence *in situ* hybridisation (FISH). FISH combined with retrograde labelling determined which colon-innervating cells express TRPV4. Quantitative RT-PCR determined TRPV4 mRNA expression in whole DRG, and specifically in laser captured colonic DRG cells identified by retrograde labelling. To determine the role of TRPV4 in colonic mechanosensation, mouse colonic splanchnic or pelvic afferents were recorded *in vitro* [1] from TRPV4+/+ and −/− mic. **Results:** FISH revealed only 21.5±0.9% of T10-L1 DRG neurons and 18.7±0.8% of L6-S1 DRG neurons expressed TRPV4, whereas TRPV4 was localised in 64.5±4.2% of colonic T10-L1 neurons and 58.1±2.0% of colonic L6-S1 neurons (both $P<0.0001$ *vs* non-colonic, n=6). Correspondingly, QRT-PCR showed greater TRPV4 mRNA expression in laser-captured colonic cells than in whole DRG. In response to graded mechanical stimuli, colonic splanchnic afferents from TRPV4−/− mice had reduced stimulus-response functions compared with TRPV4+/+, in serosal afferents by 50% ($P< 0.0001$, 2-way ANOVA, n=15), and in mesenteric afferents by 30% ($P<0.001$, n=8). TRPV4−/− also had reduced mechanosensitivity of colonic pelvic serosal afferents by 57% ($P<0.0001$, n=4). These deficits were greater than those we observed in ASIC3−/−[2]. **Conclusions:** Although TRPV4 is expressed at low abundance in whole DRG, it is prevalent in cells innervating the colon. In keeping with these results, targeted deletion of *trpv4* decreases responsiveness of splanchnic and pelvic colonic afferents, implicating TRPV4 in visceral mechanosensation. Gene products of *trpv4* may represent novel targets to treat mechanical hypersensitivity such as occurs in IBS. 1) Brierley, Gastroenterology, 127 (2004) 166–78. 2) Page, Gut, 54 (2005) 1408–15. 3) Liedke, Cell-Mol-Life-Sci. 62 (2005) 2985–3001. Supported by NHMRC Australia.

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Gastric distension-induced blood pressure changes in conscious rats

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Introduction: It has previously been shown that intragastric balloon distension increases blood pressure in healthy humans as well as in cats, pigs and rats. However, all animal studies were performed during anaesthesia. Telemetric monitoring of blood pressure provides a methodology for studying cardiovascular responses in conscious animals. We set out to study blood pressure during gastric distension in conscious Sprague Dawley (SD) and Wistar Kyoto (WKY) rats. The latter strain is known to have impaired gastric accommodation compared to SD rats. **Material & Methods:** All experiments were performed in 5 weight-matched female SD and WKY rats that were habituated to Bollmann cages in which the animals were placed during the experiment to mildly restrain them. Two weeks before the start of the experiments telemetric blood pressure devices and gastric fistulas were implanted. After an overnight fast, a test-meal infusion system and a catheter to measure intragastric pressure (IGP) were connected to the gastric fistula. The viscous, non-nutritious test-meal contained 3% hydroxypropyl methylcellulose. Following a 15 min stabilisation period the test-meal was infused until 20 mmHg IGP was reached. The change in mean arterial pressure (MAP) during infusion is expressed as % increase vs. the average MAP during 5 minutes pre-infusion. One experiment per rat was performed every 2nd week until 4 experiments per rat. MAP and IGP are expressed as mean±SEM. **Results:** In general, MAP was constant in SD rats up to

10 ml test-meal infused (IGP: 11.3±0.8 mmHg) but an increase in MAP was seen with further test-meal infusion. During the 1st experiment, maximal MAP increase in SD rats was 125±5% at 16.7±0.9 ml infused (IGP: 17.2±1.2 mmHg). This MAP increase progressively decreased for every subsequent experiment to a maximal MAP increase of 114±3% during the 4th experiment. In WKY rats MAP was generally constant up to 6 ml infused (IGP: 16.1±1.1 mmHg) but increased thereafter to a maximum of 122±4% at 9.0±0.7 ml infused (IGP: 19.3±1.02 mmHg) in the 1st experiment. As in SD rats the maximal MAP increase progressively decreased to a maximum increase of 109±2% in the 4th experiment.**Discussion & Conclusion:** For the same infused volume, both the increase in IGP and blood pressure were higher in WKY rats compared to SD rats. This suggests that WKY rats are more sensitive to gastric volume, possibly as a consequence of their impaired gastric accommodation. The decreased blood pressure response to gastric distension for every performed experiment indicates an adaptation process of an unknown origin.

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Painful colorectal distensions evoke long term memory in membrane properties of DRG neurons

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Introduction: Alteration in nociceptive sensory processing such as post-inflammatory plasticity in dorsal horn pre and postsynaptic elements has been well described. It has also known that repeated painful distension, even in the absence of overt experimentally induced inflammation, can induce sensitization of this pathway, leading to visceral hypersensitivity 24 hr later. Dorsal root ganglion (DRG) neurons projecting to the distended region will be activated during each inflation and are thus a potential site for use-dependent somatic plasticity. This was tested directly by recording from rat DRG neurons 24 hr after repeated nociceptive colorectal distension. **Methods:** Results were taken from 64 adult male Sprague-Dawley rats. Repeated colorectal distension (80 mm Hg, 30 s on, 30 s off) was performed for 1 h in 27 rats; 37 were controls with 0 mm Hg. DRG neurons were harvested after the distension and cultured. Whole cell patch clamp recordings using KMeSO4 rich pipettes were made from isolated DRG neurons 24–30 hr after the distensions. Neurons projecting to the colorectal region were identified by retrograde transport of DiI. Passive and active membrane parameters were measured in voltage recording mode. Cross-sectional area of labeled neurons was measured from captured digital images. Statistics are given as mean ± S.E.M. **Results:** Painful distension increased total membrane capacitance (mean ± S.E.M) from 49 ± 4 to 67 ± 6 pF (P = 0.03). Other passive membrane characteristics such as membrane polarization (-51 ± 2 vs. -52 ± 2 mV) and leak conductance (0.50 ± 0.15 vs. 0.47 ± 0.10 nS/pF) were unaffected. Cell cross-sectional area was increased from 1041 ± 57 to 1514 ± 100 μm^2 ($P < 0.001$). In addition, distension reduced action potential half width (2.5 ± 0.3 to 1.8 ± 0.2 ms, P = 0.04) but increased its upstroke speed (104 ± 10 to 141 ± 15 m/s, P = 0.05). The slow afterhyperpolarization was not altered. **Discussion:** These results demonstrate that repeated visceral nociceptive stimuli can leave a lasting memory trace in DRG neurons. The changes are consistent with an increase in Na and decrease in K currents. Analogous action potential and size changes occur after somatic peripheral inflammation suggesting that nociceptive activation and inflammation may access similar memory-like mechanisms. The results also raise the possibility that when inflammation is paired with painful functional stimuli, sensitization of the pain pathways may be exaggerated.

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Colitis is associated with increased excitability of sympathetic prevertebral ganglion neurons which contributes to upper bowel dysmotility

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Colitis is associated with dysmotility of not only the inflamed large bowel but also the non-inflamed small bowel. Postganglionic sympathetic neurons located in the prevertebral ganglia (PVG) provide ongoing inhibitory sympathetic tone to the entire gastrointestinal (GI) tract and receive a robust innervation from mechanosensory intestinofugal afferent neurons mostly located in the distal colon. Because these neurons provide information regarding the physiological state of the distal bowel to the rest of the GI tract, we hypothesized that they are involved in the disturbances in small bowel motor function during colitis. We have used the trinitrobenzene sulfonic acid (TNBS) model of colitis, and intracellular recording techniques to evaluate changes in the electrical properties of inferior mesenteric ganglion (IMG) neurons of the guinea pig. IMG neurons exhibit evidence of hyperexcitability 24 hours post-TNBS administration, six days post-TNBS, during the ''chronic'' phase of inflammation, as well as 56 days post-TNBS when intestinal inflammation has resolved. At the 24-hour time point, while the resting membrane potential and input resistance remain unchanged, there was a decrease in the intracellular current necessary to elicit an action potential (rheobase) (control: 351 ± 41 pA, n=15; TNBS: 217 ± 35 pA, n=13; $P<0.05$, t-test) and an increase in the number of action potentials elicited by 2.5x rheobase (control: 3.0 ± 0.2, n=15; TNBS: 6.0 ± 1.3, n=13; $P<0.05$, t-test). Small bowel transit of a semi-solid charcoal test meal 24 hours post-TNBS was reduced compared to control animals (control: 87 ± 6 %, n=3; TNBS: 45 ± 4 %, n=3; $P<0.05$, ANOVA). Pretreatment of colitic animals with the $\alpha 2$ adrenergic receptor antagonist, rauwolscine (1μmol/kg, i.p.), enhanced transit compared to non-treated colitic animals (62 ± 5 %, n=3, $P<0.05$, ANOVA). These data indicate that enhanced sympathetic drive as a result of hyperexcitable PVG neurons contributes to reduced small bowel motor function during colitis. Because hyperexcitability of PVG neurons persist following recovery from inflammation, there are likely compensatory mechanisms to restore normal gut function. Supported by DK17632 and DK17238.

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Suppression of external anal sphincter activation during sacral nerve root stimulation in pigs

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Background and aim: Neurological disorders e.g. spinal cord injury can cause disordered defecation. Sacral nerve stimulation is an alternative approach when conventional treatment fails. Electrical stimulation of sacral nerve roots to induce defecation simultaneously activates the rectum and the external anal sphincter (EAS) which obstructs defecation. The aim of the present study was to electrically activate small-diameter motor fibres in sacral roots innervating the rectum, without activating the large-diameter motor fibres to the EAS. **Materials and methods:** After spinal laminectomy the ventral portion of one S2 root was trapped in a tripolar cuff electrode in ten Göttingen minipigs anaesthetized with chloralose. A laboratory stimulator provided rectangular current pulses of 250μs, at 15Hz, in trains of 5s. Similar shaped pulses of 600μs and 427μs from the laboratory stimulator and a custom made implantable stimulator were applied to hyperpolarize the nerve cell membrane under the anode of the stimulation electrode. This was to arrest the action potentials in large nerve fibres to the EAS, leaving action potentials unaffected in small fibres to the rectum. Records of

anal and rectal pressure were obtained. In five animals, the mass of evacuated rectal contents was recorded with artificial material. **Results:** In all ten animals the anal and rectal pressure increase was the highest when stimulating the S2 ventral roots. Anodal blocking was achieved in 8 animals with the laboratory stimulator. Using pulses of 250μs the anal sphincter pressure increased to a mean maximum of 22 cmH_2O with increasing stimulus amplitude. Rectal pressure increased in a similar fashion to a mean of 30 cmH_2O. Using 600μs pulses, the current amplitude was raised above the level that gave maximum sphincter pressure. With this, sphincter activation was suppressed by a mean of 92 percent. The implantable stimulator was tested in five animals all resulting in anodal blocking. Using 427μs pulses the increase in sphincter pressure was 27cmH_2O and suppression of the pressure by a mean of 93 percent was reproduced by further raising the current amplitude. The minimum current for maximum sphincter suppression and rectal response (0.9mA) was the same for the laboratory stimulator and the implant. Evacuation of the rectal content was complete in all five animals tested. **Conclusion:** With long pulses, the laboratory stimulator as well as the implant, could suppress the EAS response by 90 percent indicating propagation arrest in the large nerve fibres to the EAS. This was achieved without impeding evoked rectal response, allowing evacuation of rectal content. **Conflicts of Interest:** This research was funded in part by MED-EL Medical Electronics, Austria.

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Characterization of T9-T10 spinal neurons with duodenal input and modulation by gastric electrical stimulation in rats

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Gastric electrical stimulation (GES) has been suggested as a therapy for some patients with morbid obesity or gastrointestinal motility disorders. However, it is unclear how GES affects sensory and motor functions of gastrointestinal tract. Also, little is known about intraspinal visceroreceptive transmission and processing for duodenal afferent information. The aims of this study were to characterize responses of thoracic spinal neurons to duodenal distension and to examine the effects of GES on activity of these neurons. Extracellular potentials of single T9-T10 spinal neurons were recorded in pentobarbital anesthetized, paralyzed, ventilated male rats (n=19). Graded duodenal distension (DD, 0.2–0.6 ml, 20 s) was produced by water inflation of a latex balloon surgically placed into duodenum. One pair of platinum electrodes (1.0–1.5 cm apart) was sutured onto the serosal surface of the lesser curvature of the stomach. GES with four sets of parameters was applied for one minute: GES-A (6 mA, 0.3 ms, 40 Hz, 2s on, 3s off), GES-B (6 mA, 0.3 ms, 14 Hz, 0.1s on, 3s off), GES-C (6 mA, 3 ms, 40 Hz, 2s on, 3s off), GES-D (6 mA, 200 ms, 12 pulses/min). Of 117 spinal neurons, 7 (6%) neurons were identified as low-threshold responses to DD (≤0.2 ml) and 26 (22%) as high-threshold responses to DD (≥0.4 ml). DD-responsive neurons were encountered more frequently in deeper (depth: 0.3–1.2 mm) than in superficial laminae (depth: <0.3 mm) in dorsal horn of spinal cord (24/67 vs 9/50, $P<0.05$). DD excited all 9 superficial neurons. In contrast, 24 deeper neurons were excited and 4 neurons were inhibited by DD. Activity of DD-responsive neurons was affected more frequently with GES-C (13/15, 87%) than GES-A (6/16, 38%), -B (3/15, 20%), -C (5/14, 36%), $P<0.01$. Bilateral cervical vagotomy did not significantly alter effects of GES on 6/6 neurons. Resiniferatoxin (2.0 μg/kg, i.v.), an ultrapotent agonist of vanilloid receptor-1, abolished DD responses and GES effect on 4/4 neurons. Additionally, 29/33 (88%) DD responsive neurons received inputs from somatic receptive fields on the back, flank, and medial/lateral abdominal areas. It is concluded that GES mainly exerts an excitatory effect on T9-T10 spinal neurons with duodenal input transmitted by sympathetic afferent fibers expressing vanilloid receptor-1; spinal neuronal responses to GES are strengthened with an increased pulse width and/or frequency of stimulation; T9-T10 spinal neurons may process input from duodenum and mediate effects of GES on duodenal sensation and motility. (Supported by OCAST)

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Identification and roles of molecules that attract or repel vagal sensory axons innervating the developing gut

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The vagal innervation of the gut is formed during fetal life. In order to establish appropriate connections between the brain and bowel, vagal sensory axons need to find their correct enteric destinations. Vagal sensory axons express the netrin receptor, deleted in colorectal cancer (DCC) and are attracted by netrins, which are expressed in the outer gut mesenchyme and endoderm. Despite the endodermal secretion of an attractant, vagal sensory axons do not enter the endodermal layer. We therefore tested the hypothesis that a mucosally secreted molecule such as laminin, or another known chemorepellent system, such as Slit [ligand]/Robo [receptor] might repel axons from specific regions of the bowel wall. DiI was applied to the nodose ganglia of E12–E16 mice to identify vagal sensory axons in the developing gut. These axons were found to descend to the stomach at E12 as fasciculated paraesophageal bundles, which defasciculated by E16, extending fine fibers into the wall of the stomach and small intestine. These DiI-labeled axons approached, but did not cross sites of laminin concentration. At E12–13, cells that expressed laminin β1 transcripts and laminin immunoreactivity extended centrifugally from the developing gut endoderm. At E15–16, cells that expressed laminin α1 transcripts and laminin immunoreactivity were found in the mucosa and outer gut mesenchyme. Neurites from explanted E14 nodose ganglia extended preferentially towards netrin-1 secreting 293-EBNA cells; this attraction was blocked by soluble laminin ($p < 0.05$) and by the amino acid sequence YIGSR (laminin β1; $p < 0.05$). Transcripts encoding Slit1 were detected in the fetal and adult stomach and small intestine; moreover, mRNA encoding Slit1 was found in developing E16 enteric ganglia by *in situ* hybridization. Transcripts encoding Robo1 were present in developing and adult nodose ganglia. DCC-expressing vagal sensory axons are attracted to sources of netrin-1 in the developing gut; laminin may terminate this attraction. Slit1 is present in the fetal bowel and Robo1 is expressed by vagal sensory axons; these molecules may further refine netrin/laminin-mediated guidance. These observations are consistent with the hypothesis that both chemoattraction (netrin/DCC) and chemorepulsion (laminin, Slit/Robo) participate in establishing the enteric vagal sensory innervation. Supported by FDHN, CDHNF, NS12963 and NS15547.

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Mechanisms of dietary free glutamate sensing by the rat gastric vagus

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Background and Aim: Recent advancements in molecular biology in the field of taste perception have raised the possibility for ingested nutrients to be ''tasted'' in the upper gastrointestinal tract. We have been the first to find that the gastric branch of the vagus nerve in the rat responded to the intra-gastric administration of the monosodium salt of glutamic acid, which is a major component of proteins in foodstuffs. That leaded us to hypothesize that the stomach could ''taste'' ingested nutrients through chemical sensing systems similar to the one functioning in the tongue and intestine. In this study, we have tried to identify the mechanism involved in the luminal glutamate sensing by the gastric vagus. **Methods:** For electrophysio-

logical recordings, male Sprague-Dawley rats were anesthetized with urethane and the nerve responses of the rat vagal gastric branches to 20 amino acids were monitored. Metabotropic glutamate receptors (mGluRs) were imunostained in thick frozen sections, fixed with 4% paraformaldehyde, with a fluorescent dye-conjugated seconday antibody (Alexa Fluor 488-labeled anti-rabbit IgG).**Results:** Afferent fibers of the gastric branch increased their firing rate solely with the intragastric application of the amino acid glutamate. Other amino acids failed to activate the gastric afferents. This response to glutamate has shown to be blocked by the depletion of serotonin (5-HT) and inhibition of the 5-HT_3 or nitric oxide synthase enzyme. Whereas, sodium nitroprusside, a nitric oxide (NO) donor, was activated the afferent nerves when applied over the stomach lumen. On the other hand, the inhibition of the 5-HT_3 abolished this NO activity over afferent fibers. Immunohistchemistry revealed a specific fluorescent signal at the apical membrane of cells from the glandular stomach. **Conclusions:** These results strongly suggest the existence of a sensing system for glutamate in the rat gastric mucosa. Luminal glutamate through the activation of mGluRs would evoke the activation of afferent fibers from the gastric vagus nerve through the production of mucosal bioactive substances (NO and 5-HT). Assuming there is a universal co-existence of free glutamate with dietary protein, a glutamate sensing system in the stomach could contribute to the gastric phase of protein digestion

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Cortical plasticity of swallowing oral muscle induced by ventilation and swallowing tasks

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Background: In stroke patients swallowing rehabilitation is the main treatment of swallowing disorders. Nevertheless, it has never been demonstrated if such rehabilitation could enhance plasticity of swallowing muscle cortical areas. The aim of our study was, in healthy subjects, to appreciate changes in cortical representation and cortical excitability induced by ventilation or swallowing tasks. **Method:** In nine healthy right handle subjects (5 females, age range 20–26 yrs), surface mylohyoid EMGs and pharyngeal pressure were recorded. Non focal transcranial stimulation (nfTMS) and focal stimulation (fTMS) of MH were performed, permitting to measure MHMEPs and modification in pharyngeal pressure. Thresholds were also measured during nfTMS and fTMS. During focal stimulation, cortical area was determined for the right MH muscle (number of point spaced by 2 cm able to product MH MEP). During nfTMS, MHMEP were realised during expiration, swallowing and sniff manoeuvre. This was performed initially and one week later to assess the reproducibility. Thereafter, subjects were asked to realise 10 minutes, everyday during one week, ventilation with glottic movements or swallowing tasks. Each subjects made the trials in a random order. After each week, the subjects were re-evaluated. **Results:** Swallowing tasks increased the cortical representation of MH muscles, as ventilation did not (Swallowing trial: n=13±7 points (p=0.05), Ventilation trial: 11±5 points (ns)). Amplitudes of the MH MEPs and amplitudes of pharyngeal pressure induced by nfTMS increased after ventilation movements and not after swallowing tasks. They were highest during swallowing. **Conclusion:** swallowing task and ventilation movements modified cortical representation of swallowing oral muscles, the first one increased cortical representation and facilitation as. the second one could modify long term plasticity.

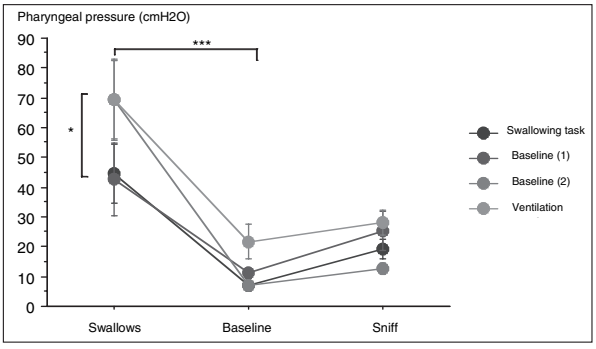

Figure 1 Modification of pharyngeal pressure after the different trials.

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Role of TRPV1 in initiation and maintenance of Visceral Hypersensitivity in a Rat Model of Irritable Bowel Syndrome

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Background: Visceral hypersensitivity is a hallmark of many functional bowel disorders but the molecular mechanisms involved in its initiation remain unknown. The transient receptor potential vanilloid 1 channel (TRPV1) is a key molecule in the activation and sensitization of sensory neurons and is expressed by most colonic extrinsic afferent neurons. Although up-regulation of TRPV1 expression has been noted in patients with IBS, little is known about its ability to initiate or to maintain long-lasting visceral hypersensitivity in this or other functional bowel disorders. We addressed this question in a rat model of IBS based upon neonatal exposure of the colon to a non-injurious solution of acetic acid. As adults (8–12 weeks), these rats display hypersensitivity to graded colorectal distention (CRD) in the absence of structural or histological abnormalities. **Aims:** Our aims were to determine whether neonatal treatment with the TRPV1 agonist capsaicin would lead to the development of persistent sensitization and whether treatment of hypersensitive adults with TRPV1 inhibitors prior to neonatal acetic acid treatment reduced persistent sensitization to CRD. **Methods:** Ten days old rat pups received a colonic infusion of 0.2 mls of 0.5% acetic acid or 50 μg/ml capsaicin or vehicle and were tested for sensitivity to CRD at 8 weeks of age. A portion of the acetic acid and vehicle treated rats were treated with 2.6 mg/kg TRPV1 antagonist SB366791 prior to testing. Responses to CRD were measured by observation of the abdominal withdrawal reflex (AWR) and by electromyographic (EMG) measurements of visceromotor responses obtained from electrodes implanted in the external oblique muscle. **Results:** Rats that received 50 μg/ml CAP as neonates were significantly more sensitive to CRD compared to both unmanipulated and vehicle treated rats at 20 ($p=0.007$), 40 ($p<0.001$), 60 ($p<0.001$) and 80 ($p<0.001$) mm Hg. SB-366,791 treatment caused a significant decline in the mean responses of P10 acetic acid treated rats to CRD at 40 mm Hg ($p=0.023$) and 60 mm Hg, ($p=0.021$) with a trend towards significance at 20 mm Hg ($p=0.094$). No significant differences were observed at 10 mm Hg, $p=0.444$ or 80 mm Hg, $p=0.224$. SB-366,791 treatment produced no significant effect upon the responses of P10 saline treated rats to CRD (10, $p=1.000$, 20, $p=1.000$, 40, $p=0.652$, 60, $p=1.000$, 80, $p=0.375$). **Conclusion:** These data suggest a critical role of TRPV1 activation in the development and maintenance of persistent visceral hypersensitivity. We speculate that changes in colonic pH in the neonatal period could activate TRPV1 and result in IBS in susceptible individuals.

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Mast cell activation sensitizes vagal afferent C-fiber's responses to mechanical and chemical stimulation in the guinea pig esophagus

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Peripheral sensitization of visceral sensory afferents is important in visceral hypersensitivity. We previously showed that antigen-induced mast cell (MC) activation potentiated vagal C-fiber's mechano-excitability through histamine H1-receptor in the Guinea Pig (GP) esophagus. The mediators of this effect, other than histamine, and whether mast cell activation affects the chemical excitability of C-fibers are unknown. **Aims:** to determine I) the mechanism underlying potentiation of mechano-excitability and 2) the effect of MC activation on the response of esophageal vagal afferent C-fibers to chemical stimulation

with P2X receptor agonist. **Methods:** GP (100–300 g) were actively sensitized by ovalbumin (OVA, ip, 10mg/kg). Beginning 21 days after the last injection the esophagus was removed along with the vagus nerve and nodose ganglion and mounted in an in-vitro organ bath system. Action potentials in vagal nodose afferent C-fibers were recorded extracellularly before and after OVA perfusion. Applying esophageal distention and a P2X agonist, α, ß-methylene-ATP, determined mechanical and chemical sensitivities of these C-fibers. The total numbers and subtypes of MCs before and after degranulation were compared by immuno-staining MC tryptase and chymas. **Results:** OVA challenge immediately potentiated vagal C-fiber's response to esophageal distension with a doubling of the peak discharge frequency evoked by 10, 30, 60 mmHg distension pressures (4±1, 8±2, 12±2 vs. 9±2, 17±5, 22±5 Hz/second, respectively P<0.05). This effect persisted for 2 hours as measured in 30-minute intervals. Pyrilamine (H1-receptor antagonist) inhibited this mechano-excitability but only if applied BEFORE MC activation. When pyrilamine was given 30 minutes AFTER OVA perfusion, the potentiation of mechano-excitability was unaffected (n=4, P>0.1). MC activation also potentiated vagal C-fiber's response to α, ß-methylene-ATP (peak spike frequency: 2±0.5 vs. 3.3±0.7 Hz, n=6, P<0.05). OVA significantly induced MCs degranulation (44±12 vs. 12±4/mm2 cross-sectional area, n=8, P<0.05) to release tryptase and chymase. **Conclusion:** ssEsophageal MC activation potentiates vagal afferent C-fiber's responses to both mechanical (distension) and chemical (P2X receptor agonist) stimuli, and thus provides a novel model to study the mechanism of peripheral sensitization of visceral sensory afferents. That Histamine H1-receptor antagonist fails to inhibit the prolonged increase in mechano-excitability once MCs are activated indicating that, although histamine is essential, there is an additional mediator involved in the pathway. We speculate involvement of other mast cell mediators such as tryptase through protease-activated receptor-2 (PAR2) mechanism.

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Abdominal distension in the Irritable Bowel Syndrome (IBS): Differences between hypo-, normo- and hyper-sensitive patients

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IBS patients with a history of bloating can be divided into those who distend (ie exhibit an increase in abdominal girth) and those who do not (1). Those who bloat alone have been shown to be more viscerally sensitive than those who bloat and distend (2). The aim of this study was to examine whether abdominal distension differs between IBS patients who are hypo-, normo- or hyper-sensitive to balloon distension. **Methods:** Abdominal girth was recorded for 24 hours using the validated objective technique of Ambulatory Inductance Plethysmography (1) in 70 IBS patients (Rome II) with a history of bloating, aged 18–73 years (39 IBS-C, 26 IBS-D, 5 IBS-alt, 62 female) and 44 healthy volunteers, aged 18–67 years (42 female). Within 7 days of this recording, rectal sensitivity was assessed using a barostat technique, in which pain thresholds were determined using the ascending methods of limits followed by tracking. **Results:** Compared with our departmental 95% normal reference range for the sensory threshold for pain of 24–38 mmHg (3); 22 (32%) patients were found to be normo-sensitive, 31 (44%) hyper-sensitive and 17 (24%) hypo-sensitive to distension. Hypo-sensitive patients distended significantly more (change in girth from beginning to end of day: +7.8cm (6.2, 9.4) cm, mean (95% CI)) than both normo-sensitive (+4.0cm (2.1, 6.0) cm; p=0.01) and hyper-sensitive patients (+3.1cm (1.7, 4.5) cm; p<0.001). In addition, significantly more of the hypo-sensitive patients (14/17) distended beyond the normal reference range of > 6.9cm than either normo- (9/22; p<0.01) or hyper- sensitive (5/31; p<0.01) patients. Although there was no significant difference in diurnal change in girth between hyper- and normo-sensitive patients (p=0.42), fewer of the hyper-sensitive patients exhibited changes in girth beyond the normal reference range than those who were normo-sensitive (p=0.06). **Conclusion:** These results show for the first time that IBS patients who are viscerally hypo-sensitive are more likely to exhibit the greatest diurnal changes in abdominal girth (ie distend). Furthermore they confirm our previous preliminary observations that hyper-sensitivity is more likely to be associated with the symptom of bloating alone (2). Refs: (1) Houghton et al, Gastroenterol 2006 (in press); (2) Lea et al, Gastroenterol 2003; 124: A-398; (3) Lea et al, Aliment Pharmacol Ther 2003; 17: 635.

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Relationship between abdominal pain and bowel frequency in functional constipation

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Abdominal pain is a frequent complaint in patients with functional constipation, but the relationship between abdominal pain and bowel frequency, if any, is controversial. Aim of this study was to evaluate the after treatment time-course of abdominal pain and the relationship, if any, between improvement of bowel frequency and abdominal pain in functional constipation. Twenty-eight patients (F:23; mean age: 41±15 yrs) seeking medical advice for functional constipation as defined with Rome II criteria[1] were interviewed by means of a standardized questionnaire, before, and 6–12 months after, a standardized treatment with bran, (~30g/day), water supplement (1,5 ml/day), high fiber diet (~15g/day) and bowel training. The presence of at least 3 spontaneous evacuations per week, without assuming laxatives, was defined normal bowel frequency. **Results:** After treatment, frequency of bowel movements became normal in 16 pts (57%) and abdominal pain disappeared in 13 pts (46%). Abdominal pain disappeared in 9 (56%) of the 16 patients with normal bowel frequency after treatment. **Conclusion:** These data suggest that a reduced bowel frequency, and possibly fecal stasis, can account for abdominal pain only in a subgroup of patients with functional constipation. Thompson WG et al. Functional bowel disorders and functional abdominal pain. Gut 1999; 45(suppl II): II43.

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Mucosal colonic mediators of patients with irritable bowel syndrome evoke increased dorsal root ganglia Ca^{2+} mobilization: Role of histamine, prostaglandins and proteases

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Background: Activated mast cells near colonic nerves correlate with abdominal pain in irritable bowel syndrome (IBS) (Barbara et al Gastroenterology 2004;126:693–702). However, the mechanism through which mast cells may affect visceral sensory perception in IBS remain unknown. **Aim:** To study the effect of the mast cell mediators histamine, prostaglandin(PG)E_2 and proteases released from the colonic mucosa of IBS patients on Ca^{2+} mobilization in rat dorsal root ganglia (DRG). **Methods:** Twenty-two Rome II confirmed IBS patients (M/F=7/15; 40.5±9.5 years old) and 10 healthy controls (HC; M/F=4/6; 24.7±3.3 years old) were studied. Colonic mucosal mast cells were identified immunohistochemically and mediators (i.e., histamine, tryptase and PGE_2) were assayed with EIA in the supernatant of mucosal biopsies incubated for 20 min. Intracellular Ca^{2+} measurements were obtained from cultured rat DRG neurons before and after HC or IBS sample exposure. **Results:** Mucosal mast cells were increased in IBS (>180%, *vs* HC, P<0.001) along with a 2–3-fold increase in histamine (P=0.015), tryptase (P<0.01) and PGE_2 (P<0.01) release vs. HC. Exogenous capsaicin (1 μM) evoked a prompt increase in $[Ca^{2+}]_i$ in 64% of the tested DRG neurons. Both HC and IBS supernatants stimulated a prompt increase in $[Ca^{2+}]_i$. Mucosal supernatants obtained from IBS patients evoked a significantly higher $[Ca^{2+}]_i$ increase *vs*. controls (29 ± 4 % *vs*. 11 ± 4 %; P<0.05). The increase in $[Ca^{2+}]_i$ obtained with IBS samples was significantly correlated with the area of the *lamina propria* occupied by mast cells (r = 0.78; P<0.01). The histamine-1 receptor antagonist pyrilamine (0.1 μM), or the protease

inhibitor FUT-175 (50 μg/ml) reduced the magnitude of the $[Ca^{2+}]$-increase evoked by IBS supernatants as compared to their vehicles (14 ± 3 % vs. 29 ± 4 % or 18 ± 5 % *vs*. 38 ± 4 %, respectively; $P<0.05$). The PGE_2 receptor antagonist AH-6809 (5 μM) had no significant effect. **Conclusions:** Mediators released from the colonic mucosa of IBS patients evoked enhanced responses in DRG nociceptive neurons, which are dependent on histamine and protease receptors.

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The diagnostic value of Rome II criteria in irritable bowel syndrome: a preliminary study on Iranian patients

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Background and aims: Diagnosis of irritable bowel syndrome (IBS) is based on symptom evaluation and the ruling out of organic disturbances. The need for simple and valid diagnostic criteria has led to the search of clusters of positive symptoms that are characteristic for IBS patients. There are still scanty data on IBS patterns in Iran which is mainly due to unavailability of a valid symptom based questionnaire in our region. The value of Rome II criteria in diagnosis of IBS as a screening method in Iranian population has been investigated in this study. **Methods:** One hundred and ninety two consecutive patients from an out-patient setting (group I) and 618 randomly selected university students (group II) were analyzed in this study. In our outpatient setting, the diagnosis of IBS was made by gastroenterologist based on ruling out organic disorders by paraclinical investigations. The diagnosis of IBS in the university students was based on Rome II criteria. The former patients' group was assessed for fulfilling the Rome II criteria (i.e. the questionnaire was the same as what has been used in the university students). Moreover, the clinical and paraclinical investigations were performed for evaluation of the definite diagnosis of IBS among the latter group. **Results:** In the outpatient setting, 116 out of 192 patients were female (60.4%). The mean age of these patients was 40.5 years (range: 16–70 years). Sensitivity of Rome II criteria was 96.3% and 92% in male and female population, respectively. In the university students, 128 (20.7%) fulfilled Rome II criteria. The mean age of these patients was 22 years (18–30 years). After investigations, 114 students had definite diagnosis of IBS. The frequency of IBS was similar among male (44/195) and female (70/423) students (P>0.05). Positive predictive value of Rome II criteria was 89% and 88% in male and female population, respectively. **Conclusion:** Rome II criteria can distinguish satisfactorily between organic diseases and IBS. To confirm the expediency of such criteria as a screening tool, further studies on community samples are recommended.

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Sensitivity measurement and subgrouping of subjects using electronic analogue scale

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Currently, the most common way of investigating sensitivity in IBS is to use ascending methods of limits, AML, to record the thresholds for discomfort and pain, and use these thresholds as measures of sensitivity. The apparent drawback of this method is that the intensity of the sensation is not taken into account.

The aim of the study was to map both IBS patients and healthy subjects onto a sensitivity index scale using a ramp-tonic colorectal distension model during which discomfort was continuously recorded with an electronic analogue scale (EAS), and then subgroup them accordingly.

We used an EAS to record discomfort and pain continuously during rectal distension. An isobaric ramp-tonic distension paradigm was used in which a 50% EAS discomfort level (of worst thinkable discomfort) triggered the transition from ramp to tonic phase. In our minds, a sensitivity index should not only reflect the threshold of sensation but also take into account the intensity of the sensation. We selected three variables from the colorectal ramp-tonic distension experiments: the maximum pressure reached during the distension; the threshold for discomfort; and the intensity of sensation measured as maximum discomfort felt during the distension.

Using these variables, we categorized 57 healthy subjects using k-means clustering into three groups: the hypo-, normo-, and hypersensitive group respectively. In each group, the mean values for the variables specify a point, called a centroid, in the space spanned by the three variables. The sensitivity index was defined based on these three centroids. The index was set to zero at the normosensitive centroid, increasing linearly to 100 in the direction of the hypersensitive centroid, and from zero to –100 in the direction of the hyposensitive centroid. Projecting the observations onto these two imaginary lines gave the value of the sensitivity index. An index in the range –50 to 50 was considered as normal, whereas an index below –50 and above 50 was classified as hypo- and hypersensitivity, respectively.

Results: Calculation of sensitivity index on all 167 (97 IBS and 70 HS) distensions available, showed that for IBS subjects the distribution in the hypo-, normo-, and hypersensitive groups was 7%, 40%, and 53%, respectively. The corresponding distribution for healthy subjects was 13%, 46%, and 41%. This method illustrates an adequate way of subgrouping both IBS and healthy subjects according to visceral sensitivity in which both threshold and intensity of the sensation is taken into account.

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Impaired accommodation in the rectum. A possible pathophysiological mechanism for irritable bowel syndrome (IBS)

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It is consistently reported that the majority of IBS patients demonstrate visceral hypersensitivity during rectal distention. But the pathophysiological mechanism behind this finding is still not clear. We propose an impaired rectal accommodation as a possible pathophysiological mechanism for IBS.

Aim: To determine whether IBS patients have abnormal rectal accommodation during rectal distension compared to healthy volunteers. **Method:** An iso-baric ramp-tonic rectal distension paradigm was used in 18 female HV and 12 IBS (8 ♀) patients. The pressure was linearly increased from 1 to a maximum of 70 mmHg in 3 min. The length of the iso-baric tonic phase was 3 min, and then the pressure was set to zero. The volume changes during the ramp-tonic distension were continuously monitored. The volume (accommodation volume) and the rate of the volume increase (accommodation rate) during the tonic phase were used as parameters for accommodation. Discomfort and pain sensations of the subjects were continuously recorded by means of a newly developed electronic analogue scale and were fed back to the distension device. At 50% discomfort the ramp phase was changed to the tonic phase. The subjects were not aware of this change. This means that the length of the ramp phase was different for each subject. **Results:** The HV as well as the IBS patients could be divided into hypo-, normo- and hypersensitive subjects (HV: n= 4, 6, 8 resp. and IBS: n= 0, 6, 6, resp.). During the ramp phase:1. the accommodation rate (AR) (ml/s) was not significantly different between the normosensitive HV and normosensitive IBS patients; 2. the accommodation volume (AV) (ml) was not significantly different between the normosensitive HV and normosensitive IBS patients. During the tonic phase the AR (0.60 vs. 0.12 ml/s, P<0.0015) and AV (59.8 vs. 21.4 ml, P< 0.0001) of the hypersensitive HV were significantly greater than for the normosensitive HV. In contrary, there was no difference between the AR (0.28 vs. 0.17 ml/s, P=0.09) and AV (36.2 vs. 22.3 ml, P=0.06) between IBS normosensitive and hypersensitive patients. This indicate that hypersensitive IBS patients has an impaired accommodate as compared to HV. We hypothesize that the impaired rectal accommodation in IBS patients will lead to symptoms in daily live **Conclusion:** Impaired rectal accommodation in IBS patients, resulting in an increase in rectal pressure during activation of the defecation reflex initiated by introduction of faeces into the rectum, can be the cause of symptoms of discomfort in these patients.

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A ramp-tonic rectal distension/electronic analogue scale paradigm to dissociate irritable bowel syndrome patients from healthy volunteers

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Altered rectal perception during distension tests is regarded as a main characteristic of irritable bowel syndrome (IBS) patients and was even proposed as a biological marker. Different distension paradigms are used but till now it is still not clear which optimal distension paradigm should be used for testing discomfort and pain. In the literature there is consensus that up to about 45% of IBS patients are hypersensitive for rectal distension using the ascending methods of limit paradigm. It is known that the pharmacology for phasic and tonic distensions is different. This is why we designed a distension paradigm, which combines both phasic and tonic distension. **Aim:** To investigate if a new paradigm of iso-baric ramp-tonic rectal distension with continuous recording of sensations could discriminate group's healthy volunteers (HV) from IBS patients. **Methods:** An iso-baric ramp-tonic rectal distension paradigm was used in 69 HV (43 ♀) and 97 IBS patients (66 ♀). The pressure was linearly increased from 1 to a maximum of 70 mmHg in 3 min. The length of the tonic phase was 3 min, and then the pressure was set to zero. Discomfort and pain sensations of the subjects were continuously recorded by means of a newly developed electronic analogue scale and were fed back to the distension device. At 50% discomfort the ramp phase was changed to the tonic phase. The subjects were not aware of this change. This means that the length of the ramp phase was different for each subject. **Results:** The area under the curve for discomfort and pain during the ramp and tonic phase was different between HV and IBS (P=0.0008 and P<0.0001, respectively). Even during the tonic phase there was a significant difference for discomfort and pain between HV and IBS (P<0.0003 and P< 0.0001, respectively). Ten seconds after the end of the tonic phase (no stimulus) discomfort and pain was still significantly different between HV and IBS (P=0.004 and P<0.0001). Fifty three percent of the 97 IBS patients showed hypersensitivity to rectal distension. **Conclusion:** One short lasting (10 minutes) iso-baric ramp-tonic distension in combination with continuous sensitivity measurements for discomfort and pain, using an electronic analogue scale, is a powerful method to measure sensitivity in subjects during colorectal distension. This technique enables clear group differentiations between healthy volunteers and IBS patients.

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Pseudoaffective response to colorectal distension in mice: Is it a physiologic indicator of visceral perception?

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Background/Aim: Visceral hypersensitivity has been implied as one of the possible mechanisms underlying functional bowel disorders, such as Irritable Bowel Syndrome (IBS). In animal models, the pseudoaffective response to colorectal distension is widely used to investigate visceral hypersensitivity. However, concerns have recently been raised on the value of abrupt, phasic distensions to mimic *in vivo* distension of the gut. Our aim was to assess different distension protocols and compare the effect of phasic versus ramp distensions in the measurement of pseudoaffective response in mice. **Methods:** Ten female NIH Swiss mice implanted with chronic EMG electrodes were examined on 3 occasions 4 days apart. Abdominal muscle EMG was recorded with a newly developed mouse barostat using isobaric or isovolumic protocols. Phasic distension consisted of 10 min distensions of 30 and 60 mm Hg with 5 min periods of rest. Ramp distension was performed during 50 seconds to a maximum of 50 mm Hg (isobaric) or 300 μl (isovolumic) with 5 mm Hg or 30 μl increments, respectively. AUC was calculated for each distension level. In ramp distension, the sensory threshold response was identified. **Results:** Both phasic and ramp distension induced a dose-dependent response in EMG. Phasic distension induced a high amplitude response that reached its peak during the first second of stimulation. Ramp distension produced lower amplitude, but more homogenous responses throughout the distension periods. First EMG responses (sensory threshold) occurred at 20 mm Hg and 150 μl, respectively. Inter-individual variability of EMG responses was significantly reduced during ramp, compared to phasic distension. **Summary:** Stepwise ramp distension produces lower magnitude but more homogenous EMG responses than phasic distension. Sudden onset of distension during a phasic distension may startle the animal and result in initially high EMG responses that are artefactual. We conclude that ramp distension is more accurate to determine the pain perception and may therefore be more suitable to study visceral hyperalgesia and allodynia in animal models.

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Decrease of postprandial serum motylin level may contribute to dyspeptic symptoms development in ESRD patients

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Background: Motilin is an intestinal peptide known mainly as a hormone of interdigestive GI tract motor activity which also affects postprandial motility. GI tract peptides release and their metabolism are impaired in renal failure patients. Disturbed motilin activity may therefore contribute to the development of dyspeptic symptoms observed with increased frequency in patients with end stage renal diseas (ESRD) treated with peritoneal dialysis (PD), as compared to general population. **Aim:** To evaluate fasting and postprandial serum motilin levels in patients with ESRD treated with PD and their correlation with dyspeptic symptoms reported by these patients. **Methods:** 33 patients (18 male, 15 female) of age 37–86 years were investigated. The motilin level was measured in blood samples taken after an overnight fasting and one hour after standard meal (Nutridrink; Ovita Nutricia) ingestion. The serum motilin activity was quantified with the application of RIA method. All patients were interviewed as regard to dyspeptic symptoms occurence with locally developed questionnaire. The results of both kinds of meaurements were analysed statistically and their correlation was evaluated. **Results:** The mean fasting motility level in patients with CRF was 201,9 + 113,5 and postprandial 319,4 + 180,4 pmol/l. No differences were obserwed between male and female patients. Postpradial motilin increase was observed in 26 patients (78,8 %) and decrease in 7 patients (21,2 %). Patients reported following dyspeptic symptoms: bloating (10 pts), belching (8 pts), esophageal regurgitation (8 pts), postprandial nausea (6 pts) and vomiting (1 pt) or heartburn (6 pts). The mean fasting nor postprandial motilin level correlated with the dyspeptic symptoms. However in patients with meal-related nausea, regurgitation and vomiting, the postprandial motilin drop was more likely to occur than in patients without these symptoms – the motilin activity decrease was observed in 8 of 15 patients. **Conclusions:** In some patients from studied group postprandial serum motilin level decrease was observed, instead of expected increase. This phenomenon was more common in patients who reported postprandial esophageal regurgitation, nausea and vomiting. We conclude that inappropriety low level of serum motilin following the meal ingestion, may adversely affects upper GI tract motility and therefore contribute to frequent posprandial dyspeptic symptoms occurence in patients with ESRD treated with PD.

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Effects of noradrenergic arousal on regional cerebral activity

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Background: The locus coeruleus (LC)-noradrenergic system (LC-NE) plays a prominent role in arousal. Functional alterations in this system have been implicated in the enhanced perceptual response of IBS patients to visceral stimuli. The bidirectional interactions between the LC-NE system and the amygdala-corticotropin-releasing factor (CRF) system play a crucial role in generating central stress responses. The NE effect on brain function has been conceptualized as increasing the

gain of cortical networks. Heightened arousal can facilitate behavior but over-arousal can lead to distractibility and anxiety. Activity of the LC-NE system can be increased by administration of the α_2 antagonist yohimbine (YOH), and decreased by the α_2 agonist clonidine (CLO). **Aims:** Identify brain regions implicated in central NE-mediated arousal and determine to what extent activity in these regions is associated with self-reported anxiety. **Methods:** Cerebral glucose metabolism [18F] fluorodeoxyglucose positron emission tomography) was assessed during performance of an audiovisual selective attention task in 7 male subjects in a cross-over, double-blind, randomized design after administration of YOH, CLO, and placebo on three different days. Subjective anxiety was measured using the UCLA Stress Symptom Rating scale. **Results:** YOH treatment was associated with increased activity in the left amygdala as compared to either CLO (t=5.01) or placebo (t=8.17). Anxiety was positively correlated with activity in the amygdala, and negatively correlated with activity in rostral anterior cingulate (rACC). **Conclusions:** These results show that activation of the amygdala is a key component of NE-mediated arousal. Subjective anxiety was positively correlated with amygdala activity, but showed a negative correlation with rACC, a region implicated in feedback inhibition of the amygdala. Supported by NIH grants DK48351, P50DK64539 and R24 AT002681(EAM), NR04881(BN).

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Increased cytokine production by peripheral blood mononuclear cells in IBS is associated with somatisation

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Introduction: The irritable bowel syndrome (IBS) is characterized by chronic or recurrent abdominal pain or discomfort along with altered bowel function. It is associated with multiple non-gastrointestinal symptoms. The aetiology is unknown although psychological factors are important and low-grade mucosal inflammation and immune activation have been reported. **Aims:** The primary aim was to assess differences in peripheral blood mononuclear cell (PBMC) cytokine production between IBS patients and healthy controls. The secondary aim was to assess if cytokine levels related to psychological and somatic symptoms. **Methods:** 34 IBS patients satisfied Rome II criteria for IBS. 14 Diarrhoea-predominant IBS, 6 Constipation-predominant and 14 Post-Infective IBS. 21 normal healthy controls (HC) were also recruited. Anxiety, Depression and Somatisation scores were recorded. PBMCs were incubated for 24 hours and supernatant cytokine levels were assayed by cytometric bead array. **Results:** Anxiety, Depression and Somatisation were significantly higher in all IBS subtypes compared to HC. PBMC incubations revealed significantly higher TNF-α, IL-1β and IL-10 production by IBS subgroups compared to HC, $p<0.05$. There was a significant association between Somatisation scores and IL-10 production, $p<0.05$. **Conclusion:** IBS differ from healthy controls in producing elevated TNF-α, IL-1β and IL-10 from PBMC incubations, suggesting that some IBS patients have an inflammatory component to their illness. Somatic symptom scores correlated with IL-10 production though whether the association is cause or effect remains uncertain.

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Mast cells in human, rat and mouse gut are 5-HT$_4$ receptor-immunoreactive (IR)

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Introduction: 5-HT$_4$ receptors were recently localized to different cell types in the human GI tract, including smooth muscle cells and mast cells. In the current study we further investigated the localization of 5-HT$_4$ on mast cells in human, rat and mouse gut, in addition to various mast cell lines. **Methods:** Sections of formalin fixed paraffin embedded (FFPE) human, rat and mouse small and large bowel were stained using immunohistochemistry (IHC) and *in situ* hybridization (ISH). The human samples were non-involved regions of resection specimens obtained with consent. Rat and mouse samples were from normal and *Nippostrongylus brasiliensis* (Nb) infected animals. Two anti-human 5-HT$_4$ antisera (NLS 656 [Novus, CO] and LS 655 [Life-Span, WA]) were employed for IHC, and 30mer biotinylated oligonucleotide probes were used for ISH. Double-stains of 5-HT$_4$ with c-kit (CD113; Dako, CA) and tryptase (Calbiochem, CA) were also performed. Cultured mast cells, including primary mouse bone marrow mast cells (BMMC), rat basophil leukaemia cells (RBL) were also FFPE and stained, in addition to the human mast cell line KU182 and wild type / 5-HT$_{4b}$-transfected CHO cells. **Results:** Mast cells in all levels of the gut and mast cell lines consistently exhibited 5-HT$_4$-IR, although the level of staining varied between the different cell types and methods, as illustrated in the table below:Mast cells in human gut samples were also double-labelled with LS655 / tryptase and LS655 / c-kit. NLS 656 antiserum produced some non-specific staining of CHO cells but this did not affect the interpretation of mast cell immunoreactivity. **Conclusions:** Mast cells in human small and large bowel, and normal and Nb-infected rodent bowel express strong 5-HT$_4$-IR. Staining was also observed in mast cell lines from all three species. These data raises the possibility that 5-HT$_4$ ligands used to modulate gut motility might act, at least in part, by modulation of mast cell function.

	Human	Rat	Mouse	BMMC	RBL	KU182	CHO	5-HT$_4$-CHO
NLS 656	+/−	+/−	+	+	+/−	+	+	++
LS 655	++	++	+++	++	+	++	-	++
ISH	+/−	N.D.	N.D	+	+	+	N.D.	N.D.

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Patients with clinically different symptom-based diagnosis of functional bowel disorders may have identical specific and non specific ileo-colo-rectal microscopic abnormalities

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Patients with endoscopy negative symptom-based diagnosis of functional bowel disorders (BD) may present specific and non-specific microscopic abnormalities that can affect the mucosa of one or more sites of the large bowel and terminal ileum.

Aim: of this study was to evaluate whether the clinical presentation of the BD differs according to the site affected by, and the specific or non-specific type of, the microscopic abnormality. **Methods:** Thirty-four consecutive patients (22 F; age range 22–70 yrs), with normal serum biochemistry, CBC, thyroid function, EMA, and normal ileum-colonoscopy were evaluated. Sixteen patients matched the symptom-based diagnosis of IBS, five of functional diarrhea (FD), and thirteen of unspecified functional bowel disorders (UFBD). Biopsies of the terminal ileum (n=2) cecum (n=1), ascending (n=1), transverse (n=1), descending (n=1), sigmoid (n=1) colon, and rectum (n=1) were stained with H-E for microscopic assessment by a pathologist unaware of the clinical diagnosis. **Results:** Histological abnormalities were found in 9 (26.5%) patients: 3 IBS, 2 functional diarrhea and 4 UFBD. Increased lymphoplasmacytic infiltrate and granulocyte clusters were present at the level of a) the ileum only in 1 IBS patient, and in 2 UFBD patients; b) the cecum only in 1 IBS patient. Altered crypt architecture was present in the rectum only in 1 UFBD. Collagenous colitis was present in 1 IBS, 2 functional diarrhea, and 1 UFBD, patients. **Conclusions:** Non-specific microscopic abnormalities of the ileo-colonic-rectal mucosa and specific microscopic collagenous colitis may be

equally present in patients with different clinical presentation of symptom-based diagnosis of functional bowel disorders.

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Sleep disturbance is an independent predictor of chronic upper and lower gastrointestinal (GI) symptoms: A population-based study

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Studies in tertiary referral patients reported an association between sleep disturbances and functional bowel disorders. Disturbed sleep patterns have been associated with altered GI motor function. We hypothesized that sleep disturbance is a risk factor for chronic upper and lower GI symptoms in the general population. **Methods:** A random sample of Olmsted county, MN was mailed a 48-item questionnaire with validated measures of key upper and lower GI symptoms of dyspepsia, gastroesophageal reflux (GERD), irritable bowel syndrome (IBS), specific questions on sleep disturbance (e.g. waking up several times during the night), quality of life and depression (exercise, smoking, alcohol use). Adjusted OR for individual symptoms due to sleep disturbance was calculated with multiple logistic regression adjusting for age, gender, depression and lifestyle confounders. **Results:** 3292 subjects responded (47%, mean age 53, 49% female). Aggregate upper and lower GI symptom scores were significantly correlated with sleeping scores ($r=0.42$, $p<0.0001$). OR (95% CI) for the association between sleep disturbance and GI symptoms are shown in the Table. Trouble staying asleep and insufficient sleep were significantly associated with increased odds for upper and lower GI symptoms, independent of gender, age, lifestyle factors and depression. **Conclusion:** In the general population, poor sleep is associated with symptoms of dyspepsia, GERD and IBS. Sleep disturbance may be relevant to functional GI disorders' pathogenesis.

Upper Symptoms	OR (95% CI)	Lower Symptoms	OR (95% CI)
Pain/discomfort in upper abdomen	1.3 (1.1–1.4)*	Diarrhea	1.3 (1.1–1.4)**
Feeling of food staying in stomach	1.2 (1.0–1.5)*	Urgency	1.2 (1.1–1.4)**
Nausea	1.2 (1.0–1.3)*	Loose, watery stools	1.3 (1.2–1.5)**
Vomiting	1.1 (0.9–1.2)	Constipation	1.1 (1.0–1.4)
Dysphagia	1.1 (1.0–1.3)*	Lumpy, hard stools	1.0 (0.9–1.2)
Acid regurgitation	1.2 (1.0–1.3)*	Fecal incontinence	1.1 (0.9–1.2)
Heartburn	1.1 (1.0–1.3)		
Bloating	1.1 (1.0–1.3)		

* $p<0.05$, ** $p<0.01$

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A meta-analysis of the placebo response in complementary and alternative medicine trials of Irritable Bowel Syndrome

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Background: Patients with irritable bowel syndrome (IBS) demonstrate a high placebo response rate in clinical trials of conventional medical therapy. IBS patients also frequently use complementary and alternative medicine (CAM), which may act through an ''enhanced placebo effect.'' The purpose of this study was to estimate the magnitude of the placebo response rate in CAM trials for IBS and to identify factors that influence this response. **Methods:** We performed a systematic review and meta-analysis of randomized, placebo-controlled clinical trials of CAM therapies for IBS identified from MEDLINE/EMBASE/PsychLIT databases from 1970 through June 2004. Placebo responses and active treatment responses for global symptom improvement were assessed. **Results:** 14 studies met the inclusion criteria. The pooled placebo response rate was 41.2% (95% confidence interval, 31.3% to 51.0%). Significant heterogeneity existed across trials (range 15.0% to 70.0%, $p<0.001$). However, we did not identify any factor that explained this heterogeneity. **Conclusion:** There is a high placebo response among IBS patients in CAM trials that is similar in magnitude to that seen in conventional medicine trials. This suggests that the placebo response is independent of the type of therapy used and that it is not particularly 'enhanced' in CAM trials. **Acknowledgement:** This research was partially funded by NIH grants #1R01 AT01414 (NCCAM-NIDDK) and # 1R21 AT002860-01 (NCCAM-OBSSR).

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Fluvoxamine versus amitryptiline in IBS female patients with depression

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Background and aim: IBS still needs a perfect therapy. Depression is a common disorder in IBS. Several trials showed the benefit of antidepressants in IBS. We looked for the effect of a SSRI, fluvoxamine vs a TCA, amitryptiline in depressed females with IBS. **Methods:** Subjects: 40 female pts with IBS filling the Rome II criteria and with depression scores on the Beck Depression Inventory were selected in a tertiary center. Protocol: The pts were randomized in 2 groups: group I received fluvoxamine 50 mg at bedtime 4 weeks; group II received amitryptiline 25 mg at bedtime 4 weeks. Both groups were comparable in respect to age and depression score. Pts were advised to avoid other medication for digestive symptoms during the trial. The effect of the drugs was assessed for 4 main symptoms with a symptom score looking for intensity and frequency of symptoms according to a scale from 0 to 4, thus allowing values from 0 to 16. (Dumitrascu et al. Health Dig Matters 2004). **Results:** From the 40 pts, 36 finished the study (18 in each leg), the other quit due to insatisfaction with the study drugs. Symptom score for abdominal pain was decreased from 11.9±3.3 to 5.6±3.6 ($p<0.02$) in group I and from 12.5±4.0 to 6.2±4.5 ($p<0.05$) in group II. Bloating score was improved from 8.8±3.3 to 5.2±2.1 ($p<0.05$) in group I and from 7.7±2.7 to 4.6±3.2 in group II ($p<0.05$). Diarrhea and constipation scores were not significantly changed. BDI scores were similarly decreased by both drugs, from 22.5±6.8 to 14.5±6.1 in group I ($p<0.01$) and from 24.2±5.9 to 16.0±7.2 in group II ($p<0.01$). Less side-effects were recorded in group I vs group II (dry mouth: 2 vs 6 cases, dizzines: 2 vs 4 cases, somnolence 5 vs 9 cases). **Conclusions:** Fluvoxamine and amitryptiline similarly improve pain and bloating as well as depression scores in females with IBS and depression. However fluvoxamine produces less secondary effects but there number is still high.

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What general practioners know about IBS in Romania

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Background and aim: IBS is a common disorder in Romania. A recent survey showed that about 2% of the subjects enrolled on the records of GPs have diagnosed IBS. Up to 30% of digestive complainers referred by GPs to tertiary centers have functional GI disorders and mainly IBS. It is necessary therefore to assess the knowledge of GPs on IBS and to find out how they manage this condition. **Methods:** One hundred GPs were invited to a 2 hours course on IBS. The course was organized in 2 venues situated at 100 km distance, at each were invited 50 GPs. The course was given by an expert and was free of charge. The course included data on epidemiology, pathogenesis, diagnosis and therapy. 88 GPs attended the course, 36 in one venue, and 52 in the other venue. All were asked to fill a questionnaire before and after the course. The questionnaire included beside questions about name initials (to retrieve the respondents before and after the course), about experience, age and sex, following multiple choice questions: What is IBS in your opinion: motility, functional or psychiatric disorder; How is established de diagnosis: based on history, on personal experience, on colonoscopy, other way; If diagnostic criteria are used; If yes, which one: Manning, Kruis, Rome I, II or III; Do they know the Rome II criteria? Have they attended previous educational programmes on IBS? How common are IBS pts in their practice: < 1%, 1–10%, 11–25%, 26–33%, >33%. **Results:** Full responses to both questionnaires were obtained from 88 GPs. They were 25 males and 63 females, with working

experience between 3 to 41 years (mean±SD: 24±11 years). Except 2 (2.4%) who indicated that IBS is a motility disorder, all GPs considered IBS as a functional disorder. All (100%) opted for the necessity of colonoscopy to diagnose IBS. All except 1 (1.2%) who said he uses Rome III criteria indicated the use of Rome II criteria for the diagnosis of IBS (this survey was done before the availability of Rome III but their advent was mentioned in the course). All (100%) indicated they know the Rome II criteria. All (100%) participated to previous lectures or courses on IBS and FGID. 58 (66%) reported the prevalence of IBS cases in their practice between 1–10%; 16 (18%) between 10–25%; 11 (12.5%) between 25–33%; 3 (3.5%) reported <1%. The 2 wrong answers on IBS as motility condition were corrected after the 2 hours course. **Conclusions:** Romanian GPs seem to be well trained to recognize IBS and report the prevalence of this condition 1–10% in their practice.

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Rapid early phase gastric emptying may be a unique characteristic of Cyclic Vomiting Syndrome: A comparison with Functional Vomiting

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Background: We have previously described that patients with cyclic vomiting syndrome (CVS) have a more rapid early-phase gastric emptying when compared to the norm. However, it is unknown if this finding is a unique characteristic of patients with this rare and poorly understood disorder. Hence, we compared the early phase gastric emptying between CVS and other vomiting disorders, specifically that of functional/psychogenic vomiting (FV). **Aim:** To determine the differences in early phase gastric emptying in patients with CVS and FV. **Methods:** A ten-year (1993–2002) retrospective review of medical records was conducted on patients diagnosed with ''Cyclic Vomiting Syndrome'' and ''Functional/Psychogenic Vomiting''. Inclusion criteria were all patients diagnosed at age 18–85 years, both male and female. Patients with concomitant medical diseases (e.g. diabetes, liver disease, etc.), previous abdominal surgeries, or on medications that may affect gastric motility (e.g. narcotics, etc.) were excluded from the analysis. Gastric emptying was performed by scintigraphy (^{99m}Tc-egg meal). Gastric Emptying at 1 and 2 hours (GE1h and GE2h) were obtained as proportion of isotope emptied (%). Wilcoxon test was used to compare differences between groups. **Results:** A total of 91 patients met inclusion/exclusion criteria; 46(23M/23F) and 45(17M/28F) patients were diagnosed to have CVS and FV, respectively. Of these, 22 CVS patients and 22 FV patients underwent gastric emptying study. There were no significant differences in gender, age of onset (25±2 vs. 31±2, in yrs.), and BMI (25±1 vs. 24±1, in kg/m^2) between CVS and FV patients, respectively. GE1h and GE2h were significantly more rapid in CVS patients compared to FV patients (GE1h: 44±5 vs. 24±4, p<0.01, % isotope emptied in 1 hour; GE2h: 72±4 vs. 59±4, p<0.04, % isotope emptied in 2 hours). **Conclusions:** CVS patients have more rapid early phase gastric emptying compared to FV patients. Rapid early gastric emptying may be a characteristic that is unique to this rare disorder. Further physiologic studies investigating this interesting finding should be pursued.

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Myoelectric and autonomic nervous system activity in patients with Irritable Bowel Syndrome

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Introduction: The pathogenesis of biopsychosocial model of functional gastrointestinal disorders (FGIDs) is still not well recognized. Explanation of the main causes of symptoms and dysfunctions in FGIDs is focused on visceral hypersensitivity. Sympathetic stimulation increases sensitivity of visceral receptors. Thus overall influences of the autonomic nervous system are important not only in regulation of secretion and motility of the gastrointestinal tract but also in visceral sensation. **THE AIM** of this study was to evaluate gastric motility disturbances and changes in autonomic nervous system (ANS) activity in irritable bowel syndrome patients (IBS). **Patients And Methods:** 10 pts (46 ± 8 yrs) and 30 healthy volunteers matched with age and gender (47 ± 5 yrs) were included in the study. For clinical evaluation first six main dyspeptic symptoms were scored from 0–3. In IBS pts symptoms mean score value was 16+/−8, in the control group 4+/−3. After an overnight fasting, autonomic activity (by assessing the heart rate variability (HRV) with CNSystem equipment) with simultaneous gastric myoelectric activity (Synetics Medical multichannel electrogastrography (EGG)) in basal conditions and after a standard meal (Enrich®, Abbot Lab.) were measured in both groups. **Results:** Electrogastrography: In IBS patients fasting EGG showed dysrrhythmia 50 ± 17% vs. the control group C-11 ± 7% of recording time (p < 0.05), which was not improved significantly after meal in the patients group (57 ± 27%). Slow waves power was higher in IBS pts than in the control group: 1264 ± 820 vs. 490± 200 μV^2 (p=0.002) and in the both groups it increased after meal. Short-term HRV recording: In IBS patients we noted decrease of the parameters of the spectral domain analysis of HRV in comparison to the control group (LF – 664,1 ms^2 vs. 811,6 ms^2; HF – 422,5 ms^2 vs. 854,6 ms^2; p<0,05 respectively). LF/HF ratio increased in IBS patients (1.89 vs. 1.2 in the control group), which may indicate sympathetic over activity. **Conclusions:** Our results show changes of the autonomic system activity in patients with IBS: decreased parasympathetic and increased sympathetic activity. The patients with autonomic dysfunction had also high percentage of dysrrhythmia time. We conclude that dyspeptic symptoms in patients with autonomic dysfunction are caused mostly by high adrenergic drive resulting in dysrrhythmias and delayed gastric emptying.

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Effect of a fermented milk containing *Bifidobacterium animalis* DN-173 010 on bloating and health-related quality of life in Irritable Bowel Syndrome (IBS) adult patients - A randomized, double-blind, controlled trial

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General practitioners in France conducted the study.

Introduction and Aim: The effects of probiotics on IBS symptoms have been previously explored in small sampled studies. The present study aimed to assess the effect of a fermented milk containing a specific probiotic strain, *Bifidobacterium animalis* DN-173 010, on symptoms and quality of life (QoL) in a larger number of IBS patients. **Methods:** 267 IBS patients (199 females, 68 males; mean age 49±11 years) fulfilling Rome II criteria were randomized, after a baseline period of 1 to 3 weeks, to consume twice daily for 6 weeks either a fermented milk (Activia®, 125-g pot) containing a yogurt symbiosis and *Bifidobacterium animalis* DN-173 010 or a heat-treated yogurt containing only non-living yogurt symbiosis (control). Bloating and QoL (Functional Digestive Disorders questionnaire (FDDQL)) were assessed at baseline and at 3 and 6 weeks. Stool frequency and consistency were assessed throughout the study. **Results:** Treatment with product was associated, as compared with control, with a significant (p<0.05) reduction of bloating score after 3 weeks (product -0.56±1.01, control −0.31 ± 0.87). This effect did not remain significantly different after 6 weeks. As compared to baseline, the discomfort dimension score of FDDQL was significantly (p<0.001) improved in both groups after 3 (product 10.68 ± 14.45, control 7.52 ± 14.71) and 6 weeks (product 12.20 ± 16.18, control 13.51 ± 19.28); without difference between groups. The proportion of responders for discomfort dimension (at least 10% improvement of score vs baseline) was significantly (p<0.005) higher in the product group at 3 weeks (product 65.2% vs control 47.7%) but did not differ statistically at 6 weeks (product 63.0% vs control 56.8%). While stool

frequency and consistency did not differ between groups, a sub-analysis of those definitely constipated (<3 bowel movements/week) patients showed a significant (p<0.001) increase, as compared to control, in stool frequency over the 6-weeks product consumption. **Conclusions:** A fermented milk containing *Bifidobacterium animalis* DN-173 010 could demonstrate its ability to improve bloating and digestive discomfort of IBS patients and further research is warranted to investigate the potential of this product.

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Vagal biofeedback and breathing exercises: Promising treatment of functional dyspepsia?

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Background: Patients with functional dyspepsia (FD) have postprandial symptoms, impaired gastric accommodation and low vagal tone. The aim of this study was to improve vagal tone, and possibly thereby also gastric accommodation and symptoms, using breathing exercises with vagal biofeedback. **Methods:** Forty FD patients were randomised to treatment or non-treatment groups. The treatment was breathing exercises, 6 breaths/min, 5 minutes each day for 4 weeks, using specially designed software for vagal biofeedback. Effect variables were: maximal drinking capacity using a drink test (Toro® clear meat soup 100ml/min), intragastric volume at maximal drinking capacity using three-dimensional ultrasonography, respiratory sinus arrhythmia (RSA), skin conductance (SC) and dyspepsia-related quality of life scores (Short Form Nepean Dyspepsia Index, SF-NDI). **Results:** Drinking capacity and quality of life improved more in the treatment group than in the non-treatment group (p = 0.002 and p = 0.01) without any significant change in baseline autonomic activity (RSA and SC). Intragastric volume and gastric emptying remained unchanged in the treatment group, but compared with before, gastric emptying became slower in the non-treatment group (p=0.046). **Conclusion:** Breathing exercises with vagal biofeedback increased drinking capacity and made the patients with functional dyspepsia feel better.

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Abnormal small bowel motility in gastroesophageal reflux disease and irritable bowel syndrome

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Background: An overlap of symptoms in patients with gastroesophageal reflux disease (GERD) and irritable bowel syndrome (IBS) has recently been reported [Neurogastroenterol Mot 2005; 17: 29–34]. In both diseases, we observed abnormal motility patterns in the small intestine [Gut 2004; 36 (Suppl.1): A208, Scand J Gastroenterol 1996; 31: 581–589]. The aim of the present study was to compare the disturbances of small bowel motility in GERD and IBS patients. **Methods:** The 24-h motility data of 29 GERD patients (10 females, 19 males, aged 20–73 years) and of 49 IBS patients (32 females, 17 males, aged 22–77 years) were compared with reference values obtained in 50 healthy controls. Abnormal jejunal motility patterns observed in GERD and IBS were compared with each other using X^2 test. Small bowel motility was recorded with a portable datalogger and a nasojejunal catheter incorporating 3 miniature pressure sensors spaced at 10 cm intervals beyond the ligament of Treitz. Fasting motility and the motor response to a standardized evening meal (600 kcal) were analysed visually and by a computer program [Scand J Gastroenterol 1994; 29: 1076–1082]. **Results:** Abnormalities of small bowel motility were detected in 28 (97%) GERD and 47 (96%) IBS patients. During fasting, an abnormal motility was detected in 90% in both groups. An abnormal postprandial motility was registered in 69% of the GERD and 77% of the IBS patients. Comparing abnormal motility patterns in both patient groups, statistically significant differences (p<0.001) were observed for a disturbed phase III (48% in GERD vs. 12% in IBS patients), the occurrence of bursts (69% in GERD vs. 2% in IBS patients) and sustained uncoordinated activity (24% in GERD vs. 0 in IBS patients). **Conclusion:** Small bowel motility is disturbed in the vast majority of GERD and IBS patients. However, the abnormal motility patterns differ between both groups with higher rates of a disturbed phase III, bursts and sustained uncoordinated activity in GERD.

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Increased plasma catecholamines during sleep in women with constipation-predominant irritable bowel syndrome

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Background/Aims: Self report of disturbed sleep is a frequent complaint of patients with irritable bowel syndrome. The purpose of this study was to compare the plasma levels of norepinephrine and epinephrine during a 12 hour wake- sleep period between: 1) women with irritable bowel syndrome (IBS) and healthy Controls and 2) women with a-diarrhea-predominant IBS (IBS-D) and constipation-predominant IBS (IBS-C). **Methods:** The women (recruited from the community) were studied during their luteal phase for three nights in a sleep laboratory. The Bowel Disease Questionnaire (BDQ) was used to ascertain Rome II categorization, the Symptom Checklist-90 (SCL-90) to examine psychological distress, and the Pittsburgh Sleep Quality Questionnaire to assess subjective report of sleep quality. During the third night a venous line was inserted and blood was drawn hourly for plasma levels of norepinephrine and epinephrine (Bi-CAT radioimmunoassay). The night was divided into three phases: pre-sleep awake (8–10 PM), sleep phase (1–5 AM), and awakening (6–7 AM). Mann-Whitney non-parametric rank sum tests were used to compare groups. **Results:** Plasma norepinephrine and epinephrine values across all three phases did not differ between the 31 women with IBS and the 29 Control women (all comparisons p>.2). The 14 women with IBS-D had lower levels of norepinephrine (medians 251, 101, 157 ng/ml in the three phases) compared to the 8 women with IBS-C (302, 196, 269), only significant (p=.004) during the sleep phase. Median epinephrine levels (37, 16, 24 ng/ml for IBS-D versus 26, 32, 27 for IBS-C) were nearly 50% lower during the sleep phase in the IBS-D group than the IBS-C group, but this difference was not statistically significant (p = .156). IBS-C patients also had higher (p<.05) SCL-90 Global Symptom Index scores as compared to IBS-D and Controls (Control, 0.27; IBS-C, 0.76; IBS-D, 0.29). Twice as many women in the IBS-C group had polysomnographic-based sleep efficiency index scores less than 80% as compared to the IBS-D group (63% versus 30%). **Conclusions:** These findings are consistent with results from our earlier studies of 24-hour heart rate variability during sleep in women showing lower sympathetic/ parasympathetic balance in IBS-D than in IBS-C women.

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Utility of a modified Sitzmark study for random or serial measurement of whole gut/colonic transit time

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Aim: To assess test-retest reliability of a modified Sitzmark study with ability to assess the whole gut transit time randomly (i.e., at unpredictable times) or serially. **Methods:** Twenty-one healthy female volunteers (mean age 28.4 years), with regular menstrual cycles, were enrolled in this study. Whole gut transit time was measured twice during the luteal phase and once during the early follicular phase (menses) of their menstrual cycle. Subjects underwent two separate abdominal x-rays during the luteal phase of the menstrual cycle (three days apart) to assess test-retest reliability. Forty capsules, each containing 12 radio opaque markers, were taken (one capsule twice a day), beginning on the 14th day of their menstrual cycle. Subjects returned on the 20th and 23rd day of their menstrual cycle (six and nine days after beginning to take the markers) and on the 3rd day of

their next menstrual cycle to have an abdominal x-ray taken. The formula recommended by Metcalf and colleagues to calculate mean colonic transit time (MCT) was modified to simply counting the number of markers in the abdomen and equating these with hours of transit. This simplification is possible because we gave 12 markers every 12 hours. **Results:** Test-retest reliability was demonstrated between the 1st and 2nd transit time assessments during the luteal phase (Spearman's rho =.556, p = 0.009, n = 21). No difference was seen between the first (48.3 hours) and second (50.4 hours) mean transit times, suggesting that equilibrium was achieved (p = 0.7, paired t-test, n = 21). As predicted, test-retest reliability coefficients were greater for patients with shorter transit times (Spearman's rho = .796, p = 0.006, n = 10) than for patients with longer transit times (Spearman's rho =.334, p = 0.35, n = 10) using a median split of transit times from the first study. As expected, the third Sitzmark measurement (during menses) demonstrated faster transit (41.3 hours) than either study performed during the luteal phase. However, these differences were not significant, perhaps due to the small sample size of this pilot study. **Discussion:** This modified Sitzmark study demonstrates significant reliability and can be used to objectively document, and to prospectively study, alternating bowel habits as a subtype of IBS. In addition, the modified Sitzmark protocol may be a useful tool for investigations into the effects of drugs on bowel habits. [Supported by R24 DK67674, RO1 DK31369, and RR00046.]

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A dose-ranging, double-blind, placebo-controlled study of lubiprostone in patients with irritable bowel syndrome with constipation

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Irritable bowel syndrome (IBS) affects nearly 30 million people in North America and accounts for 25 to 50% of gastroenterology referrals. Lubiprostone, a novel type-2 chloride channel activator, has been shown to be efficacious and well tolerated in well-controlled clinical trials in patients with chronic idiopathic constipation. We present the results from a 12-week, dose-ranging study where lubiprostone was tested in patients with IBS with constipation (IBS-C), as defined by the Rome II criteria.

Methods: 194 patients were randomized and treated in a double-blind fashion with one of four treatment groups: placebo or 16, 32, or 48 mcg lubiprostone daily (8, 16, or 24 mcg BID). In a diary, patients recorded dosing, abdominal symptoms (bloating and discomfort/pain), bowel movements (BMs)–frequency, straining, and consistency ratings–and rescue medication use. Diary questions queried patients on assessment of treatment effectiveness. Trend-tests were used to detect efficacy relationships and a step-down procedure was used to make comparisons between the lubiprostone and placebo groups. Safety was assessed by adverse event (AE) incidence rates. **Results:** Significant dose-dependent trends were observed during at least 2 of the 3 months for abdominal discomfort/pain, abdominal bloating, spontaneous BM (SBM) frequency, stool consistency, bowel straining, and assessments of constipation severity. Pairwise comparisons revealed many significant differences between the active groups and placebo. During Months 1 and 2, improvements in abdominal discomfort/pain and SBM frequency rates were more than doubled in all lubiprostone groups, compared to placebo. Specifically, at Month 1, decreases from baseline in abdominal discomfort (based on a 5-point scale) were .19, .45, .40, and .46 points in the placebo, 16, 32, and 48 mcg groups, respectively; at Month 2, decreases from baseline were .23, .52, .53, and .54, respectively; and, at Month 3, decreases from baseline were .34, .56, .59, and .53 points, respectively. Overall, improvements were consistent across all active treatment arms. With respect to safety, dose-dependent trends were observed, with increased incidences of AEs and discontinuations with increasing dose. **Conclusion:** Lubiprostone is an efficacious and well tolerated treatment for IBS-C. Dose-dependent trends were observed with respect to safety and efficacy, with the 8 mcg BID group demonstrating the best profile of efficacy and safety. This research was funded by Sucampo Pharmaceuticals, Inc.

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Rectal motor physiology in diarrhea-predominant Irritable Bowel Syndrome and influence of acute serotonergic modulation

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Rectal pressure-volume relations may differ between diarrhea-predominant irritable bowel syndrome patients (d-IBS) and healthy controls. The effect of acute serotonergic modulation on rectal pressure-volume relations is unknown. Serotonergic activity can be decreased and increased by acute tryptophan depletion (ATD) and intravenous (i.v.) administration of the selective serotonin reuptake inhibitor citalopram (Cit), respectively.

The aims of the present study were to evaluate the influence of ATD and Cit administration on rectal motor physiology using a rectal barostat procedure in patients with d-IBS and controls.

Following a randomized, double blind placebo controlled crossover design an ATD and Cit experiment were conducted. The ATD experiment consisted of oral administration of 75 g of a tryptophan-free amino acid mixture and the tryptophan-containing mixture served as a control condition. The Cit experiment consisted of a single i.v. bolus of Cit 20 mg or placebo (saline). Fourteen d-IBS patients and 14 healthy matched (age, sex, BMI) controls participated in each experiment. The barostat procedure consisted of intermittent rectal distensions. Each distension lasted 1 minute, followed by 30 s baseline pressure. Rectal volume was measured at t = 10s and t = 60s after the start of each distension. Rectal adaptive relaxation (RAR) (ml/s) was measured as the delta volume per second between t=10 and t = 60s, for each distension (P = 10, 15, 20, 25 mmHg). Rectal volume (ml) at t = 60s (RV), RAR and compliance (RC) were determined. The data were analyzed using repeated measures ANOVA. **Results:** In each experiment d-IBS patients showed significantly decreased RV ($p's < 0.04$), RAR ($p's < 0.03$), and RC ($p's \leq 0.05$), compared to controls. ATD and Cit did not significantly influence RV, RAR or RC ($P's > 0.1$). **Conclusions:** D-IBS patients demonstrate disturbed rectal pressure-volume relations. However, acutely decreasing or increasing serotonergic activity does not affect these characteristics in d-IBS patients or healthy controls.

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Vagal afferents discharge and mioelectrical activity in gastric hyperalgesia in rats

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The long term exposure of gastric mucosa to inflammatory factors are suspected to alter the normal stomach motility. The consequence of it is an abnormal sensomotor response to food causing dyspeptic symptomes. Our study aimed to investigate the vagal afferents and gastroduodenal slow wave response to mild gastric mucosa inflammation in rats. The gastric mucosal inflammation was induced in rats by addition iodacetamide to drinking water for the 5 days. Slow wave and vagal nerve recordings were performed on the 6th day. Than gastric mucosa was examined. Iodoacetamide irritated gastric mucosa presented minimal inflammatory infiltration with neutrophils and mast cells. The vagal afferent activity was significantly increased after iodacetamide treatment from 0.3 ± 0.1 to 1.9 ± 0.58 Hz. The gastric slow wave frequency accelerated from 0.08 ± 0.01 to 0.1 ± 0.02 Hz ($p<0.05$) and duodenal fraction of FFT spectrum remained unchanged (from 0.64 ± 0.02 to 0.59 ± 0.1 Hz). These results suggest that mild gastric mucosa irritation sensitizes vagal afferents and alters gastric but not duodenal pacemaker activity which may contribute to dyspeptic sensations.

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Prevalence of functional gastrointestinal disorders and relevance of anxiety and depression to functional gastrointestinal disorders: A population-based study in South Korea

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Background/Aims: Even though functional gastrointestinal disorders (FGID) include common problems, their population-based data in Asia are lacking. The cause of these disorders remains unknown, but psychological abnormalities have been implicated. Thus, the aims of this study were to evaluate the prevalence of FGID and relevance of anxiety and depression to FGID in a population-based study in South Korea. **Methods:** A total of 1,774 ethnic Korean households living in Kwangju city, South Korea (818 male and 956 female, 18-94 year-old) were interviewed face-to-face by using a questionnaire comprising demographic features, the hospital anxiety-depression scale, a validated gastroesophageal reflux disease (GERD) questionnaire and the Rome II criteria for functional dyspepsia (FD), irritable bowel syndrome (IBS) and functional constipation (FC). **Results:** Among 1,774 subjects, the prevalence of GERD, FD, IBS, and FC was 9.4%, 6.0%, 10.0%, and 11.2%, respectively. Ulcer-like, dysmotility-like and mixed subtypes were observed in respectively 15%, 32% and 53% of the FD subjects. Diarrhea-predominant, constipation-predominant and mixed subtypes were observed in respectively 39%, 34% and 26% of the IBS subjects. When anxiety and depression were defined as its total scores of 11 or more, 909 (51.2%) and 1035 (58.3%) subjects had anxiety and depression, respectively. Anxiety was significantly more prevalent in female, over 40 year-old, GERD, and IBS subjects, compared with male, below 40 year-old, non-GERD, and non-IBS subjects, respectively, but not in FD and FC subjects. Depression was significantly more prevalent in over 40 year-old compared to below 40 year-old, but not in female, GERD, FD, IBS, and FC patients. The prevalence of anxiety and depression did not significantly differ between FD subtypes and between IBS subtypes. In logistic regression analysis, female (OR: 1.580), over 40 year-old (OR: 1.603), the presence of GERD (OR: 1.747), and the presence of IBS (OR: 3.379) were independently associated with anxiety. However, only over 40 year-old (OR: 1.625) was independently associated with depression. **Conclusions:** The prevalence of GERD, FD, IBS, and FC in the Korean population is 9.4%, 6.0%, 10.0%, and 11.2%, respectively. Among FGID, GERD and IBS are significantly associated with anxiety, but not with depression. The etiopathogenetic relationship between FGID and the psychological factors warrants further investigation.

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A 6 month Prognostic Model for IBS without diarrhea in primary care: The Irritable bowel syndrome Longitudinal Outcomes Study (ILOS)

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Background: Although irritable bowel syndrome (IBS) is a common condition, surprisingly little is known about the natural history of the condition. **Aim:** To develop a clinically relevant 6 month prognostic model for outcomes in IBS (without diarrhea) among patients being seen in community practices. Methods**:** ILOS was a prospective 6 month, observational study which enrolled 380 subjects from across the US who had a physician diagnosis of non-diarrhea-predominant IBS. Patient-reported data on symptoms, satisfaction with treatment, work productivity and health related quality of life were collected at baseline and at Month 6 (M6). Total Symptom Severity (TSS) was calculated (sum of freq, intensity and bother of constipation, gas, abdominal pain/discomfort and bloating; 0 to 56, lower scores are better). HRQOL was measured using the validated IBS-QOL (0 to 100, higher scores are better). Exploratory regression models identified baseline predictors of M6 TSS and HRQOL scores; predictors generally available to the clinician were preferred and the same predictors were included in both models. **Results:** At entry 93% of subjects were female with a mean age of 42 years. M6 questionnaires were available for 231 subjects. TSS improved by 24 points from BL to M6 ($p<0.01$). The models explain a significant proportion of variation in M6 outcomes (HRQOL r^2=58%, $p<0.01$; TSS r^2=27%, $p<0.01$). Clinically relevant predictors include: for HRQOL, patients with a history of Specialist Visits have mean scores 4.34 higher (better) while those with Prior Abdominal Surgery and Use of Pain Meds have scores 3.94 and 3.42 points lower (worse); for TSS, for every 10 points higher in baseline HRQOL, patients had 1.3 point lower (better) month 6 TSS scores. **Conclusion:** Our primary outcomes (symptoms and quality of life) are associated with prior specialist visits, prior abdominal surgery and use of pain medications. Although these are likely surrogate markers of disease status, they are easily obtained in a clinical practice and can be used to help guide the expectations of primary care patients for their near term outcomes. **Conflict of Interest:** This research has been funded by Novartis Pharmaceuticals Corporation.

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Results of glucose breath testing for small intestinal bacterial overgrowth (SIBO) in irritable bowel syndrome (IBS) patients: clinical profiles and effects of rifaximin treatment

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Introduction: SIBO may coexist with IBS and eradication therapy has been reported as effective in reducing IBS symptoms. **Purpose:** 1.To assess the clinical profiles of IBS patients, who underwent a breath test with a glucose substrate (GBT) 2.Evaluate hydrogen(H_2) and methane(CH_4) parameters in IBS subgroups 3.Assess if gastric acid inhibition contributes to SIBO 4.Investigate rifaximin, a non-absorbable antibiotic, for eradication and symptom relief. **Patients and Methods:** 204 IBS patients (170F&34M; mean age 46.4; range 18-88), who underwent GBT and met Rome II criteria were evaluated in this retrospective study. After an overnight fast 2 baseline breath samples were obtained followed by ingestion of 50g glucose in 148ml solution and then breath samples for H_2 and CH_4 were collected every 15 minutes for 2 hours. Positive test was a peak concentration >20ppm of H_2 and/or CH_4, or a rise >12ppm, if baseline was >10pmm. Additionally 8 IBS patients with positive GBT were treated with rifaximin 800mg per day for 1 month and assessments of symptoms and GBT performed before and after treatment. **Results:** Among all 204 IBS patients 93(45.6%) had positive GBT. 68(73.1%) of these 93 had IBS diarrhea dominant (IBS-D), 12(12.9%) were constipation dominant (IBS-C) and 4(4.3%) had alternating bowel habits. 48.6% of SIBO positive patients were receiving PPI therapy compared to 39.3% with negative GRT. 61(65.6%) produced only H_2, 27(29.0%) only CH_4, and 5(5.4%) both. There were more exclusive CH_4-producers in IBS-C than IBS-D (58.3%vs.27.9%) while in IBS-D had more H_2-formers (70.6%vs.41.6%). Those with both H_2 and CH_4 had more IBS-C (8.3%vs. 2.9%). 8 patients with SIBO received rifamixin. Repeat GBT showed that 6(75%) normalized, 1 patient (12.5%) was negative by H_2 criteria but CH_4 remained positive. Improvement in overall symptom score was observed in 7(87.5%) patients: 4 more than 75%, 2 between 50-75%; 1 between 25-50% and one had no response. No adverse events were observed. **Conclusions:** 1.SIBO was present in nearly half of this large IBS population when studied by GBT criteria 2.IBS-D is dominated by H_2-formers while IBS-C by CH_4-producers 3.Chronic PPI therapy does not appear to contribute to SIBO 4.Rifaximin is a promising, effective and safe antibiotic in treatment of SIBO in IBS although controlled trials are required.

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Symptom clusters within abdominal pain reported in a U.S. community: a factor analysis

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Background: Frequent abdominal pain (AP) is reported by 25% of people in the community. AP may result from a myriad of causes and

manifest itself in a multitude of forms but 2 disorders, irritable bowel syndrome (IBS) and dyspepsia, are thought to represent the cause of AP in the majority of patients. **Aim:** To investigate whether distinct, independent symptom clusters exist within the range of abdominal pain. **Methods:** Responses to questions from a survey of a random sample of 4196 community subjects were coded as 46 binary (0,1) variables relating to features of AP; for example, location (upper/lower), severity, duration, description (e.g. pressure, burning, crampy, relation to meals), relief in relation to bowel movements, and radiation outside the belly. A Principal Components Factor Analysis examining two orthogonal rotation methods (Varimax and Quartimax) were used to obtain a simple factor structure. As an additional check, an oblique centroid variable cluster routine was used to identify disjoint subsets of variables in which the cluster components explained as much of the original variation observed in the data as possible. Individual subject scores were computed from the identified factors and the distribution of these scores summarized overall and by a priori symptom complex definitions (e.g. Rome II IBS). **Results:** Of the 2300 respondents (55% response rate), 2255 had data available for analysis. A total of 751 (33%) indicated they experienced abdominal pain, and of these, 686 (91%) had completed all questions used to define the 46 variables. The scree plot from the factor analysis indicated 2 potential factors that accounted for approximately 19% of the variation in all 46 variables. Both rotation methods implied two factors with: 1) lower abdominal cramp-like pain, relieved by defecation, and having more or looser bowel movements when pain begins, loading high on the first factor (IBS-like: 10% variation explained), and 2) lower and upper abdominal pain, occurring 2-8 hours after a meal, relieved by belching and eating, and radiating to the back, loading high on the second factor (non-specific pain: 9% variation explained). The disjoint variable cluster approach also clustered the same variables together loading high on the first principle component factor. **Conclusion:** This factor analysis of AP in the community identified 2 factors: one consistent with IBS and the second with non-specific pain. Yet 81% of the variance remains unexplained which suggests that AP in the community is more heterogeneous than simply IBS and dyspepsia.

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Minimal change inflammation of the terminal ileum mucosa can be related to cecum-ileum reflux in patients with functional bowel disorders

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Minimal non specific change inflammation of the terminal ileum have been reported in patients with Functional Bowel Disorders (FBD) (1). Small Intestine Contrast Ultrasonography (SICUS) (2), performed after distension of small bowel lumen with oral polyethylene glycole (PEG) solution, in addition to visualize and measure in a standardized and reproducible manner the luminal diameter and the wall thickness of the small bowel, enables to visualize the direction of flow of the contrast PEG solution through the entire small bowel. In particular SICUS can detect forward and backward flux through the ileocecal valve. **Aim:** of this study was to assess in FBD patients, whether the presence of cecum-ileum reflux (CIR) was associated with histological alterations of the terminal ileum mucosa. Patients.Twenty-three consecutive patients (15 F; age range 22-63 yrs), with chronic bowel disorders, normal ileum-colonoscopy and normal: serum biochemistry, CBC, thyroid function, EMA, were evaluated after and overnight fast with ultrasonography (US) (Tosbee equipment, 3,5 MHz and 5 MHz linear transducers, Toshiba Japan) after the ingestion of 375 ml of PEG solution. Continuous US observation of the tract including terminal ileum, ileo-cecal valve and cecum was performed for 20 min and recorded on video-tape. Video-tape was later viewed in blind by the sonographist unaware of the clinical diagnosis. Two biopsies of the terminal ileum performed at endoscopy were stained with H-E for microscopic assessment by a pathologist unaware of the clinical diagnosis. **Results:** SICUS did not show any small intestine abnormality in all patients. At SICUS a cecum-ileum reflux through the ileo-cecal valve was observed in 11 patients. Lymphoplasmacytic infiltrate and granulocyte clusters were found at the level of the terminal ileum in 3 patients with CIR. No inflammatory changes were found in absence of CIR ($p<0.03$ vs presence of CIR). Isolated lymphoid hyperplasia of the terminal ileum was found in 9 patients: 4 with, and 5 without, CIR (n.s.). **Conclusions:** These preliminary observations suggest that in patients matching the symptom-based diagnosis of FBD, minimal change inflammation of the terminal ileum mucosa, and not lymphoid hyperplasia can be related to cecal contents reflux into the terminal ileum.

1) Marcheggiano et al Gastroenterology 2006; 2) Pallotta et al Lancet 1999.

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Does alexithymia play a role in the natural history of functional gastrointestinal disorders (FGID)?

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This paper aims to investigate the role played by alexithymia in the natural history of FGID through a systematic review of the literature. In the past 3 decades, a consistent body of research has shown that the psychological construct of alexithymia (difficulty in the cognitive processing of emotions) is associated with a range of disorders of affect regulation and constitutes one of the risk factors of the individual vulnerability to somatization disorders. Only few studies have however investigated alexithymia with validated measures in FGID patients. The overall findings from these studies show that alexithymia may play different roles at different phases of the course of FGID. At the preclinical stage of symptom perception, alexithymia was found to be higher in patients with FGID than patients with organic diseases (IBD and GERD), even after controlling for moderating variables. At the diagnostic stage of health care referral, alexithymia was higher in FGID patients with comorbid psychopathology referred to a gastrointestinal care setting than psychiatric outpatients with comorbid FGIDs referred to a mental health care setting, even after controlling for comorbidity. At the clinical stage of treatment response, alexithymia was a significant predictor of non-response to treatment in FGID patients, over and above symptoms of anxiety and depression. At the outcome stage of symptom maintenance, alexithymia was shown to be a significant predictor of persistence of functional gastrointestinal symptoms in patients with gallstone disease one year after surgery for cholecystectomy, over and above psychological symptoms. Different speculative hypotheses might explain these results, related to psychological (symptom amplification, attributive cognitive styles, abnormal illness behavior) as well as neurobiological (dysregulation of the prefrontal and anterior brain regions, commonly found in both FGID and alexithymic subjects) factors.

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Bacterial overgrowth in patients with irritable bowel syndrome versus diseased controls: A prevalence study

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Purpose: Recent data suggest that small intestinal bacterial overgrowth (SIBO) is commonly associated with irritable bowel syndrome (IBS). Several conditions, including Crohn's disease, small bowel resection and diabetes mellitus, are considered risk factors for the development of SIBO. The purpose of this study is to assess the prevalence of SIBO as measured by a glucose breath test in patients with IBS compared to a diseased control group. **Methods:** Thirty-six patients with IBS according to Rome II criteria underwent a glucose breath test. The mean age was 49 (19-74) years and there were 32 females (89%). The diseased control group consisted of 48 patients who had at least one predisposing condition for SIBO and who were referred for a glucose breath test. The mean age in the control group was 56 (27-85) years and there were 30 females (63%). The most common predisposing conditions in the control group were Crohn's

disease (29%), history of small bowel resection (19%) and diabetes mellitus (13%). A positive test required the elevation of hydrogen or methane by greater than 12 ppm above the baseline value after ingestion of 50 g of glucose.**Results:** Of the 36 IBS patients, 7 (19%) had positive breath tests. In the diseased control group, 30 of 48 patients had a positive test (63%). The difference between the groups was significant (OR:6.9; 95% CI: 2.5-19.0, $p < 0.001$).The mean age of the control group was significantly older than the IBS group ($p < 0.5$). Even after adjusting for age in a logistic regression model, the odds of having a positive breath test for the diseased control group remained significant (OR: 6, $p<0.001$). All IBS patients with SIBO rated their symptoms as either moderate or severe. Elevation in breath hydrogen was seen in the majority of diseased controls who had positive breath tests (26 of 30, 87%) as compared to IBS patients with positive breath tests (3 of 7, 43%). This difference between the groups was also significant ($p<0.02$). **Conclusion:** Glucose breath testing suggested the presence of SIBO in a small subset of patients with IBS. In this study, SIBO was more frequently found in patients with predisposing conditions compared to patients with IBS.

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Changes of colonic microecology as a cause of IBS

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Background: Irritable bowel syndrome (IBS) is a very commonly encountered disorder in the gastroenterologic practice. It is still interpreted as a psychosocial disease. The manifestation of the syndrome can often be related to food toxicoinfection, enterocolitis, antibiotic abuse and long-lasting usage of antacids, all of which may cause changes in colonic microecology- bacterial overgrowth syndrome. Facultative enteric pathogens are known to be producers of biologically active substances as GABA, histamine, 5-HT, CCK, etc., that may be regarded as false neurotransmitters causing polymorphic IBS symptoms. **Materials and methods:** We examined fecal microecology of 1865 patients with complaints characterized as IBS according to Rome criteria. Fecal flora was evaluated by culturing enterobacteriacea, paying attention to traditional infectious agents and facultative pathogens. Anaerobic flora was examined microscopically in stained fecal preparations quantitatively evaluating the amount of lactobacilli, bifidumbacteria, bacteroides and enterococci as agents of normal flora. We also quantitatively evaluated the amount of clostridium strains in these samples. **Results:** Changes in microecology were observed in 86.0% of the cases. Our data revealed two types of disturbances: 1. Massive colonization by facultative pathogens as Klebsiella, Proteus, Enterobacter, Pseudomonas and E. coli abnormal strains, observed in 71.9% of the cases; 2. Insufficient quantities of normal colonic flora (bifidumbacteria, lactobacilli) were observed in 82.2% of the cases. In 34.8% of the cases with insufficient normal colonic flora there was an increase in the amount of clostridium strains. The first-type changes in microecology were corrected by using antibiotics against determined enterobacteriacea according to susceptibility. The second-type changes were corrected by administering bacterial preparations containing lactobacteria and bifidumbacteria. We prescribed metronidazol or tinidazol for the patients with increased amount of clostridium strains. Improvement by 81.2%? and 87.3% was observed in 1st and 2nd group of patients respectively.
Conclusions: Our results suggest microbiological alterations in the colonic microecology of the IBS patients. These alterations can be successfully corrected by using antibiotics followed by bacterial preparations containing normal colonic flora as etiological treatment.

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Respiratory allergy and the response to the inhalant allergens skin prick test in patients with Irritable Bowel Syndrome (IBS)

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In published data, we observe that volunteers with a diagnosis of IBS reported higher reactivity to food antigens when compared to patients with functional dispepsy and normal controls (Soares RL et al. Correlation between IBS and the response to food antigens extract skin prick test. Bras J Med Biol Res 2004;37:659-62). **Aim:** the aim of this present study was to correlate respiratory allergy and symptoms of IBS with the cutaneous response to inhalant antigens using the prick test. **Methods:** We studied the response to inhalant antigens using the skin prick test in 113 volunteers who were students and employees of the Faculty of Medicine of UFF, Niteroi, Rj, Brazil. Subjects were divide into 3 groups after evaluation for ROMA II criteria for functional disease of the gastrointestinal tract: Group I, 35 volunteers with IBS(25 women and 10 men; mean age 35 years), Group II, 25 volunteers with functional dyspepsia (12 women and 13 men; mean age 29 years), Group III, 53 volunteers without habitual gastrointestinal symptoms (28 women and 25 men; mean age 32 years). The subjects were submitted to the skin prick test with 6 inhalant antigen extracts, for a total of 678 skin tests (6 per volunteer). The study was approved by the Research and Pos-Graduation Adviser Committee -CNPQ-UFF. **Results:** Of the 210 tests applied to G I, 46 (21,9%) were positive (a 3-mm wider papule than the negative control), and of the 150 tests applied to G II, 42 (21,9%) were positive. Of the 318 tests applied to G II, 69 (21,6%) were positive. There were no significant differences in the number of positive responses to prick test among the three groups (X^2-P>0,05).No difference in the number the respiratory allergic conditions was found between the 3 groups: GI, 16 (45,7%), GII, 11 (40,7%), GIII, 21 (39%) (p>0,05) and rhinitis was the most common condition. **Conclusion:** The results showed that the IBS patients have much higher cuatneous reactivity to food than to inhalant allergens.In conclusion we suggest the presence of a possible alteration in intestinal epithelial function in IBS.

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Association between inducibile nitric oxide synthase (iNOS) genetic polymorphisms and postinfectious dyspepsia: a preliminary report

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Introduction: Patients with postinfectious dyspepsia (PD) have a high prevalence of impaired accommodation attributable to a dysfunction at the level of gastric nitrergic neurons. Experimental evidence revealed that damage of enteric nitrergic neurons can occur in presence of excessive nitric oxide (NO). Infection-induced NO release is modulated by different polymorphisms of the inducibile NO synthase (iNOS), but there are no data on PD. The aim of this study was, thus, to identify the genetic polymorphisms of the iNOS gene promoter in patients with prospectively identified PD compared with unspecified-onset dyspepsia (UD) and healthy controls. **Methods:** A total of 90 consecutive patients with undergoing acute gastroenteritis were followed-up for 1 month. Patients with persistence of symptoms were studied and followed-up during 3 and 6 months to assess the evolution of dyspeptic symptoms. They underwent upper GI endoscopy with testing for Helicobacter pylori infection, gastric emptying (GE) and electrogastrography (EGG) studies. In PD and UD-patients and in 30 age-sex matched controls the genomic DNA was isolated from blood and genotyping of the pentanucleotide (CCTTT) iNOS gene promoter polymorphisms was performed by polymerase chain reaction and restriction analysis. **Results:** After the acute infection, persistence of dyspeptic symptoms up-to 6 months was observed in 20 subjects. Systematic analysis revealed that postprandial fullness, bloating, belching, nausea and epigastric pain were the most prevalent symptoms, followed by early satiety, epigastric burning and vomiting. Symptoms severity was gradually reducing from 1 to 6 months. Dealyed GE and impaired EGG were observed in 40 and 60 % of PD patients, respectively, and were similarly prevalent in UD-patients. Genotyping of iNOS gene promoter showed that PD had a different number of repeats in of CCTTT pentanucleotide (11-13 n. of repeats) compared to

UD-patients and controls respectively (14-17). **Conclusion:** We showed that at least 20% of prospectively evaluated patients with acute gastroenteritis develop postinfectious dyspepsia. These patients have symptoms profile, delayed GE and impaired EGG similar to UD-patients, but they have a different genetic polymorphisms of the iNOS. If confirmed on large series, this finding could identify a subset of patients genetically predisposed to develop persistent dyspepsia after an acute gastroenteritis.

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Subtyping irritable bowel syndrome (IBS) by the predominant bowel habit: Rome II versus Rome III

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The Rome II criteria for IBS classified patients into subtypes based on a combination of the stool frequency and form, together with the presence or absence of defecation straining and urgency. However, the recently published Rome III criteria proposes subtyping of IBS patients based on the stool form alone, and suggests that researchers use the Bristol Stool Form Scale for defining the subgroups. The concordance between subtyping of IBS patients based on Rome II versus Rome III is unknown.

Methods: We included 107 IBS patients according to the Rome II criteria (79 females; mean age 38 (19-72) years). All these patients also fulfilled the Rome III criteria for IBS. The patients completed the Rome II Modular Questionnaire to divide patients into subgroups according to Rome II, and the Bristol Stool Form Scale during one week for subtyping according to Rome III. Based on the Rome II criteria patients were defined as having diarrhea- or constipation predominant IBS (c-IBS or d-IBS), and patients not fitting into these groups as alternating IBS (a-IBS). Based on the Rome III criteria patients were divided into IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), mixed IBS (IBS-M) or unsubtyped IBS (IBS-U). **Results:** Subtyping based on the questionnaires resulted in the following groups: Rome II: 42 d-IBS, 24 c-IBS and 41 a-IBS; Rome III: 51 IBS-D, 25 IBS-C, 8 IBS-M and 23 IBS-U. Of the 42 patients with d-IBS according to the Rome II criteria 25 had IBS-D, 1 IBS-C, 3 IBS-M and 13 IBS-U based on Rome III, whereas of the 24 patients with c-IBS according to Rome II, 9 had IBS-C, 6 IBS-D, 3 IBS-M and 6 IBS-U based on Rome III. The 41 patients with a-IBS based on Rome II were divided into the following subgroups according to Rome III: 20 IBS-D, 15 IBS-C, 2 IBS-M, 4 IBS-U. As expected the stool form differed significantly between the subgroups based on Rome III, but the average stool frequency/day only differed between IBS-C and IBS-D (1.6 ± 0.99 vs. 2.7 ± 1.6 stools/day; p=0.007). **Conclusion:** Subtyping of IBS patients based on the predominant bowel habit differs substantially between the Rome II and III criteria, with a substantial proportion of patients belonging to different subtypes depending on the criteria used. The change in the definition of IBS subgroups needs further validation and might have important implications for inclusion in clinical investigations and the interpretation of past and future studies.

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The prevalence of IBS is significantly higher with the Rome III compared to the Rome II diagnostic criteria

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Background: Iterations of IBS diagnostic criteria have led to different estimates of prevalence rates. The Rome III criteria require a lower frequency of symptoms compared to Rome II (at least 3 days per month, i.e., at least 10% of the time for Rome III, compared to at least 25% of the time for Rome II). In a Rome II epidemiological survey of a representative sample of adult Israeli Jews (80% of the Israeli population), we reported a prevalence rate for IBS of 2.9% (4.1% after adjusting for alternators missed with Rome II). These low rates raised the question of whether the Rome II criteria are overly strict. The official Rome II Integrative questionnaire, used for our study, can provide a very close approximation of Rome III rates, enabling comparisons of the Rome II and III criteria. The 5 frequency response options to symptom questions in the Rome II Integrative questionnaire are: a) ''not at all or rarely''; b) ''occasionally'' (more than 10% and less than 25% of the time); c) ''often'' (up to 50%); d) ''very often'' (more than 50%) and e) ''almost always''. To meet the Rome II criteria a response of ''often'', ''very often'', or ''almost always'' is required. However, a response of ''occasionally'' (more than 10%) also meets the Rome III criteria. **Methods:** The Rome II Integrative questionnaire was validated in Hebrew. The Israel Ministry of the Interior prepared a sample of 10,000 adults. We attempted to reach 1,839 randomly selected individuals, 1,221 successfully. When 1,000 interviews were completed the survey was terminated. The overall response rate was 54% (1,000/1,839). The response rate for successfully contacted individuals was 81.9%. **Results:** The Rome II-III comparison for IBS and its sub-types appears in the table. **Conclusions:** Using Rome III criteria the prevalence rate for IBS in our population is significantly higher than by Rome II criteria (11.4% and 4.1%, respectively). The Rome III results more likely reflect the true situation in our study population compared with the overly strict Rome II criteria. Prospective studies, in this and other populations, should be conducted to confirm these results.

Disorder	Rome II	Adjusted Rome II⋆	Rome III
IBS	28 (2.9)	40 (4.1)	112 (11.4)
IBS with diarrhea	9 (0.9)	9 (0.9)	18 (1.8)
IBS with constipation	16 (1.6)	22 (2.2)	41 (4.2)
IBS-mixed	3 (0.3)	9 (0.9)	53 (5.4)

⋆Adjusted for alternators who are missed by the formal Rome II criteria.

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Efficacy and safety of lubiprostone for the treatment of chronic idiopathic constipation in elderly patients

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Constipation is a common and bothersome gastrointestinal condition that is especially prevalent among the elderly (those ≥65 years of age). Lubiprostone is a novel type-2 chloride channel activator (*Am J Physiol Cell Physiol.* 2004;287:C1173-C1183) that has been shown to be efficacious and well tolerated in three well-controlled clinical trials of 3 to 4 weeks' duration (*Gastroenterology.* 2002;122:A315, *Gastroenterology*. 2003;124:A38, *Am J Gastroenterol.* 2005;100:S324, S328, S329). We examined pooled results from these trials so that subgroups of elderly and non-elderly patients could be compared. **Methods:** In order to create an adequate pool of elderly patients, data were combined from three well-controlled clinical trials: one Phase 2 and two Phase 3 pivotal studies. Spontaneous bowel movement (SBM) frequency rates, stool consistency ratings, and bowel straining assessments were compared between treatment groups (placebo and lubiprostone 48 mcg/day) via inferential statistics and used to assess efficacy. Mean improvements over placebo were compared for elderly and non-elderly patients. Adverse event (AE) incidence rates were also compared for treatment groups and age groups. **Results:** The pooled elderly subgroup consisted of 31 placebo and 26 lubiprostone patients. Mean changes from baseline in SBM rates were significantly improved among lubiprostone elderly patients, compared to their placebo counterparts during Weeks 1, 3, and 4 ($p \leq 0.0286$). Improvements during each of the 4 weeks ranged from 4.6 to 5.4 additional SBMs per week for elderly lubiprostone patients, compared to 1.3 to 2.3 SBMs per week for elderly placebo patients. Results were similar for stool consistency and bowel straining ratings. With regard to safety, fewer elderly lubiprostone patients experienced AEs (46.2%), compared to their placebo counterparts (61.3%), a result that was reversed in the non-elderly

subgroup (65.7% lubiprostone vs. 40.1% placebo).**Conclusion:** Lubiprostone is an efficacious and well tolerated treatment for chronic idiopathic constipation in the elderly. This research was funded by Sucampo Pharmaceuticals, Inc.

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Long-term safety and efficacy of lubiprostone for the treatment of chronic idiopathic constipation in elderly and non-elderly patients

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Constipation is a common and bothersome gastrointestinal condition that is especially prevalent among the elderly (those ≥65 years of age). Lubiprostone is a novel type-2 chloride channel activator that has shown long-term safety and efficacy for the treatment of chronic idiopathic constipation in three open-labeled clinical trials (24 and 48 weeks in duration). **Methods:** In order to create an adequate pool of elderly patients, data were combined from these three studies, resulting in 163 elderly patients (age ≥65 years) and 715 non-elderly patients (age 18 to 64 years). Safety was assessed by adverse event (AE) incidence rates and efficacy was evaluated by improvements in patient assessments of constipation severity, abdominal bloating, and abdominal discomfort. All assessments were based on 5-point severity scales where 0 = Absent and 4=Very Severe. **Results:** Data revealed that, with regard to safety, slightly fewer elderly patients reported AEs, compared to non-elderly patients (121/163, 74.2% vs. 573/715, 80.1%, respectively). Notably, the incidence rate for the most common AE, nausea, was markedly decreased in elderly patients, compared to non-elderly patients (17.8% vs. 29.4%, respectively). With regard to long-term efficacy, improvements in assessments of constipation severity, abdominal bloating, and abdominal discomfort were all statistically significant at all post-baseline time points from Week 1 to Week 24 or 48 for both elderly and non-elderly subgroups ($p<0.0001$). Amongst the elderly, constipation severity was improved by an average of 1.11 points at Week 1 (n = 108), 1.32 points at Week 24 (n = 81), 1.21 points at Week 48 (n = 58), and 0.97 points for the last on-drug measurement (n = 159). Abdominal bloating was improved by an average of 0.76 points at Week 1 (n = 108), 0.99 points at Week 24 (n = 81), 1.05 points at Week 48 (N=58), and 0.71 points for the last on-drug measurement (n = 159). Abdominal discomfort was improved by an average of 0.49 points at Week 1 (n = 108), 0.75 points at Week 24 (n = 81), 0.79 points at Week 48 (n = 58), and 0.52 points for the last on-drug measurement (n = 159). **Conclusion:** We conclude that lubiprostone is a well-tolerated and efficacious long-term treatment for chronic idiopathic constipation in both elderly and non-elderly. This research was funded by Sucampo Pharmaceuticals, Inc.

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Efficacy and safety of lubiprostone for the treatment of chronic idiopathic constipation in male patients

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Constipation is a common and bothersome gastrointestinal condition, affecting nearly 30 million individuals in North America. Lubiprostone is a novel type-2 chloride channel activator (*Am J Physiol Cell Physiol.* 2004;287:C1173-C1183) that has been shown to be efficacious and well tolerated in a number of well-controlled clinical trials of 3 to 4 weeks' duration (*Gastroenterology*. 2002;122:A315, *Gastroenterology*. 2003;124:A38, *Am J Gastroenterol.* 2005;100:S324, S328, S329). We examine pooled results from these trials so that subgroups of male and female patients can be compared. **Methods:** In order to create an adequate pool of male patients, data were combined from three well-controlled clinical trials. Spontaneous bowel movement (SBM) frequency rates, stool consistency ratings, and bowel straining assessments were compared between treatment groups (placebo and lubiprostone 48 mcg/day) via inferential statistics and used to assess efficacy. Mean improvements over placebo were compared between male and female patients. Adverse event (AE) incidence rates were also compared between treatment groups and genders. **Results:** The pooled subgroup of males consisted of 27 placebo and 32 lubiprostone patients. Male patients taking lubiprostone experienced approximately twice as many SBMs per week as their placebo counterparts (5.69 to 6.05 SBMs/week vs. 2.55 to 3.23 SBMs/week, respectively). Despite the small sample size, differences were statistically significant for the first 3 weeks ($p \leq 0.0489$) and just missed significance at Week 4 ($p=0.0503$). Compared to female lubiprostone patients (N = 239), these rates were slightly higher (rates among female lubiprostone patients ranged from 4.99 to 5.75 SBMs/week). With regard to safety, one third (33.3%) of male placebo patients experienced at least one AE, compared to 50.0% of male lubiprostone patients. **Conclusion:** Lubiprostone is an efficacious and well tolerated treatment for males with chronic idiopathic constipation. This research was funded by Sucampo Pharmaceuticals, Inc.

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Lubiprostone effects on morphine-induced constipation and analgesia

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Lubiprostone is a novel and selective activator of type-2 chloride channels that is currently under development for the treatment of opioid-induced constipation (*Am J Physiol Cell Physiol.* 2004;287:C1173-C1183). In the present studies, the effects of lubiprostone on morphine-induced constipation and analgesia were examined using morphine-treated mice. **Methods:** ICR mice were initially administered 5 mg/kg morphine hydrochloride intraperitoneally, followed by an oral graphite marker. Immediately thereafter, vehicle or lubiprostone (0.1, 1, 10, 100 mcg/kg) was administered orally. Control animals received the graphite marker and vehicle without morphine. All animals were sacrificed 150 minutes after administration of the marker. Animals were then scored based on the presence of the marker found in the cecum. **Results:** Lubiprostone significantly increased the intestinal transit of the graphite marker in the morphine-treated animals, compared with that of the morphine-treated control group ($p<0.01$ at 1 mcg/kg lubiprostone or higher). The effect of lubiprostone on the analgesic effects of morphine was also evaluated. ICR mice received 5 mg/kg morphine hydrochloride intraperitoneally, followed immediately by the oral administration of vehicle or 0.1, 1, 10, or 100 mcg/kg lubiprostone. An additional control group received vehicle without morphine. At 30, 60, 90, 120, and 150 minutes after administration of morphine or vehicle, the base of the tail was pinched with forceps and the time required for a response was measured. A significant increase in response time was observed for the morphine-treated control group, compared with the normal (no morphine) control group at 30 minutes after morphine injection. In addition, all animals treated with morphine and lubiprostone responded in a manner similar to that observed with the morphine-treated control group at all time points, even at doses of lubiprostone that were 100-fold higher than those necessary to inhibit morphine-induced delays in intestinal transit. **Conclusion:** These results indicate that lubiprostone significantly ameliorates the morphine-induced delay in intestinal transit without affecting the central analgesic effect of morphine. This research was funded by Sucampo Pharmaceuticals, Inc.

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Neomycin inhibits TRPV1 mediated stress-induced visceral hypersensitivity in maternal separated rats

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Introduction: We have recently shown that visceral hypersensitivity in response to an acute water avoidance stress (WA) in maternal separated rats (MS) is TRPV1 dependant. Others have shown that aminoglycoside antibiotics like neomycin are capable of blocking the capsaicin evoked *in vitro* activation of TRPV1. Because neomycin was shown to lead to a significant reduction in IBS symptoms, we inves-

tigated the possibility that neomycin is capable of interfering with the *in vivo* stress-induced TRPV1 mediated visceral hypersensitivity in maternal separated rats.**Methods:** MS and non-handled (NH) Long Evans rats were equipped with EMG electrodes in the abdominal muscles connected to a telemetry transmitter to record the visceromotor response (VMR) to colorectal distention. Visceral sensitivity was assessed by intermittent colon distention (1, 1.5, 2 ml) before and 24 hours after WA. 30 minutes prior to post-WA distensions, rats were pretreated (i.p.) with either capsazepine (10 mg/kg), neomycin (100 mg/kg), amoxycillin (100 mg/kg) or vehicle alone. Further, we measured the distension induced VMR before and 30 minutes after intracolonic capsaicin (100 μL 0.1%) in NH rats. Another group of NH rats was pretreated with neomycin or vehicle 30 minutes prior to intracolonic capsaicin. Post-WA or post-capsaicine VMR to colorectal distention was calculated by setting the maximum value of the first distension protocol in each rat at 100%. Differences between pre- and post-WA (or capsaicine) area under the curve (AUC) were analyzed for statistical significance by Wilcoxon signed ranks. **Results:** In NH vehicle-treated animals WA did not lead to an enhanced VMR to distension, and pretreatment with capsazepine, neomycin or amoxycillin did not affect this post-WA response (n = 7/group). In MS rats all vehicle treated groups (n = 7/group) showed significantly enhanced VMR after WA (p = 0.018). Capsazepine (p = 0.237) and neomycin (p = 0.237) both prevented the stress-induced hypersensitivity, which was still apparent in the amoxycillin (p = 0.028) treated group (n = 7/group). Further, a significant increase in AUC was observed after colonic capsaicin instillation in NH rats (p = 0.018, n=7) whereas vehicle alone did not induce enhanced VMR (p = 0.612, n=7). Neomycin prevents this capsaicin induced hypersensitivity response (n=3). **Conclusion:** Acute stress triggers TRPV1 mediated visceral hypersensitivity in MS, but not in NH rats. This *in vivo* response can be inhibited by acute capsazepine and neomycin treatment, but not amoxycilline. Further, neomycin is capable of inhibiting capsaicin-induced colonic hypersensitivity. Therefore, we conclude that neomycin-mediated alleviation of visceral hypersensitivity may be partly due to its TRPV1-modulating effect.

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Serotonin transporter (SERT) activity and functional dyspepsia

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Background: Functional dyspepsia is associated with altered gastric motility and sensibility. Serotonin influences motility, and visceral perception. Consequently, variation in serotonin reuptake, caused by functional polymorphism in the gene encoding for the serotonin transporter protein (SERT), has been associated with motility specific symptoms of irritable bowel syndrome. **Aim:** To assess the association between a functional polymorphism in the gene encoding for SERT activity and functional dyspepsia. **Materials and Methods:** Consecutive symptomatic patients referred for an open access endoscopy who had a normal upper GI anatomy were eligible for enrollment. All had symptoms compatible with functional dyspepsia. Healthy controls were recruited through an advertisement in a local newspaper. Both controls and patients donated a venous blood sample and patients completed a gastrointestinal specific questionnaire . Long (l/l), short (s/s), and heterozygous (l/s) SERT polymorphisms were identified by polymerase chain reaction-based restriction fragment length polymorphisms. **Results:** Eighty-five patients with functional dyspepsia and 147 controls were included. The *s/s*, *l/l* and *s/l* polymorphisms were similar in patients and healthy controls (p>0,05). Patients were divided into 4 subgroups according to predominant symptom: ulcer-like dyspepsia, dysmotility-like dyspepsia, reflux-like dyspepsia and non-specific dyspepsia. Among patients with predominantly dysmotility-like symptoms the *l/l* genotype was more frequent, and the *l/s* genotype was less frequent when compared with healthy controls (65% vs 31%; and 12% vs 48%, respectively; $p<0,05$). **Conclusion:** Genetic SERT polymorphism appear to be unrelated to functional dyspepsia, although subgroup analysis revealed an association between the *l/l* polymorphism and predominantly dysmotility-like symptoms.

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The association between variations in the genes encoding for COMT activity and functional dyspepsia

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Background: Autonomic dysfunction may account for a wide range of functional gastrointestinal disorders. Two prominent neurotransmitters responsible for signal transduction in the autonomic nervous system are epinephrine and norepinephrine, and both are deactivated by Catecholamine-O-Methyl Transferase (COMT). COMT enzyme activity is regulated by a common polymorphism causing substantial variations in enzymatic activity. **Aim:** To assess the association between variations in the gene encoding COMT activity and functional dyspepsia. **Material and Methods:** Consecutive symptomatic patients referred for an open access endoscopy who had a normal upper GI anatomy were eligible for enrollment. All had symptoms compatible with functional dyspepsia. Healthy controls were recruited through an advertisement in a local newspaper. Both controls and patients donated a venous blood sample and patients completed a gastrointestinal specific questionnaire. DNA was extracted from whole blood and four single nucleotide polymorphisms (rs6269, rs4633, rs4818 and rs4680) in the COMT polymorphic region were determined using real-time polymerase chain reaction. Haplotypes were assigned using PL-EM version 1.0. **Results:** Eighty-five patients with functional dyspepsia (45% male; mean age (±SD) 55.8±12.6) and 147 controls (33% male; mean age (±SD) 46.6±15) were included. The frequency of COMT polymorphisms was evenly distributed among patients and controls (p>0.05). Haplotype analysis identified five separate haplotypes together representing >95 % of the study population. There was no association between haplotype distribution and functional dyspepsia (p=0.75). **Conclusion:** Genetic polymorphisms in the gene encoding for COMT activity do not confer a higher risk for symptoms in functional dyspepsia.

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Abdomino-phrenic dyssynergia, abdominal bloating and distension

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Background & Aim: We have previously shown that patients complaining of abdominal bloating have abnormal accommodation of the anterior abdominal wall to intraabdominal volume loads. Our present aim was to determine whether the diaphragm is also involved in abdominal accommodation, and whether bloating is associated to abdomino-phrenic dyssynergia. **Methods:** In 10 patients complaining of abdominal bloating (7 IBS and 3 functional bloating) and 8 healthy subjects, the volume of the abdominal cavity was increased by a colonic gas load (24 ml/min rectal gas infusion for 1 h). With the subjects sitting on an ergonomic chair (and the trunk erect) we measured abdominal girth (by a tape measure), as well as, electromyographic activity of the anterior abdominal muscles (via 4 pairs of surface electrodes) and of the diaphragm (via 6 ring electrodes over a tube positioned at the oesophageal hiatus). **Results:** In healthy subjects, the colonic gas load increased girth (by 7±1mm), increased anterior wall tone (by 15 ± 4%) and relaxed the diaphragm (activity decreased by 22 ± 5%). With the same volume load, patients developed significantly more abdominal distension (12 ± 1mm, girth increment) and this was associated to a paradoxical muscular accommodation with a relaxation of the anterior wall (activity decreased by 25 ± 8%) and a contraction of the diaphragm (by 26 ± 9%; $p < 0.05$ vs health

for all). **Conclusion:** 1) Intraabdominal volume changes are accommodated by a coordinated anterior wall/diaphragmatic response. 2) Patients with abdominal bloating exhibit objective distension and dyssynergia.

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Cyclic symptoms in patients with gastroparesis—differences from non-cyclic patients undergoing gastric electric stimulation

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Introduction: While most patients with the symptoms of gastroparesis (GP) have chronic symptoms, others have symptoms that are cyclic in nature. We investigated patients presenting with the symptoms of GP, most of whom underwent eventual placement of Gastric Electrical Stimulation (GES) to see if patients with Cyclic Symptoms differed from those with No Cyclic Symptoms. **Patients:** From a data base of 345 consecutive patients seen, 48 patients (8 m, 40 f, mean age 41.5 yrs) with Diagnosis: 18 Idiopathic, 27 Diabetes Mellitus and 3 Post-Surgical disorders, presented with the symptoms (Sx) of GP. Most patients were drug refractory and referred for possible GES. Patients were 35 (3 m, 32 f) with Cyclic Symptoms (Cyc), and 13 (1 m, 12 f) with No Cyclic Symptoms (NoCyc). 38 of 48 patients (25 Cyc and 13 NoCyc) underwent implantation of permanent GES, mean of 33 months. Results were compared by paired t-tests, baseline vs. latest for GES patients, reported as mean ± SE. **Results:** Cyc patients were similar to NoCyc patients with the exception of solid gastric emptying and health related quality of life, both of which were more impaired in Cyc Vs NoCyc patients. After GES placement (mean mo), the percentage improvement in all parameters (Sx, GET and HRQOL) was greater in Cyc than NoCyc patients. (See table below). **Conclusions:** We conclude that, based on this sample, GP patients with Cyclic Symptoms appear to respond better to GES than those with No Cyclic Symptoms. These preliminary results need confirmation in larger, prospective trials.

Baseline	Vb	Nb	TSSb	GET4b	QOLb
NoCycSx	2.4	3.3	14.2	18.8	13.9
CycSx	2.9	3.0	14.7	29.5	16.4
Betwn p	0.3	0.6	0.7	0.3	0.02

GES	Va	Na	TSSa	GET4a	QOLa
NoCycSx	2.0	3.0	11.8	13.9	11.0
CycSx	0.9	1.6	9.0	16.4	9.1

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Intestinal electrical stimulation improves delayed gastric emptying and discomfort induced by duodenal distension in dogs

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Background & Aim: Mechanical duodenal distension inhibits gastric motility and induces signs of discomfort in animals. Gastric electrical stimulation with short pulses is a novel treatment option for chronic, intractable nausea and for vomiting associated with gastroparesis. It is unknown whether such a method could be applied to intestine electrical stimulation (IES). The aim of this study was to investigate the effects of IES with short pulses on duodenal distention-induced delayed gastric emptying and discomfort in dogs. **Material and Methods:** The study was performed in six healthy female dogs implanted with two pairs of small bowel serosal electrodes, and a duodenal fistula for the assessment of gastric emptying. Each dog was studied in two sessions on two separate days in a randomized order. Each study session consisted of 20-min baseline, 60-min duodenal distension by a balloon filled with air at a volume of 40-50ml with or without IES. Gastric emptying was assessed every 15 min via the duodenal cannula for a period of 60 min by calculating the amount of phenol red mixed with the test meal (100mg phenol red in 237ml Boost) . IES was performed with pulse train with on time of 0.1 sec, off time of 4.9sec, a pulse frequency of 14Hz, a pulse width of 300usec, a pulse amplitude of 6mA. Signs of discomfort in animals were observed and noted, including licking tongue, closing eyes, yawning, rapid breathing, movement, nausea and vomiting. These signs were assessed based on their severity and/ or frequency. **Results:** (1) IES significantly improved delayed gastric emptying of liquid induced by duodenal distension. Gastric emptying at 60 min was 7.18 ± 1.99 % in the distension session, and increased to 18.05 ± 4.06 % (p =0.036, vs. distension session) in the session with IES. (2) IES significantly improved the animal behaviors suggestive of visceral pain or discomfort induced by distension. The average sign score was 15.33 ±1.37 during the duodenal distension, and reduced to 6.50±0.91 (p=0.0009, vs. distension session) in the session with IES. **Conclusion:** IES with parameters commonly used for in gastric electrical stimulation for nausea and vomiting in gastroparesis improves duodenal distension-induced delayed gastric emptying and prevents duodenal distension-induced signs of discomfort. IES with appropriate parameters may have a therapeutic potential for pseudo-obstruction in clinical settings.

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Endoscopic temporary gastric electrical stimulation—results of 146 consecutive patients

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Introduction: Gastric electrical stimulation (GES) is now an accepted therapy for drug refractory gastroparesis, but requires surgical device implantation. We recently described techniques for placement of temporary GES electrodes via PEG tube or endoscope (GIE 2005: 61:455-461). We now report on a larger series of consecutive patients referred for endoscopic temporary stimulation. **Patients:** 146 consecutive patients, 29 male, 117 female, mean age 41 years, with the diagnosis of diabetes (DM, n = 43), post-surgical (PS, n = 14) or idiopathic disorders (ID, n =88)were evaluated. We investigated those patients who were successfully implanted, and those who completed a full evaluation of symptoms and gastric emptying tests. **Methods:** All patients underwent baseline assessment of Symptoms (SX, as Nausea= N, Vomiting=V, Total GI Sx= TSS) and measurements of Gastric emptying (GET) and again after a minimum of 3 days of GES with standard parameters (GIE, above). Patients were reported by descriptive statistics and by t-tests for symptoms and emptying. **Results:** 145 (99%) of patients were successfully implanted and 130 (89%) of patients completed at least 3 days of therapy. Of the patients who completed a full evaluation, there were significant improvements in symptoms, and improvements in gastric emptying for some patients: particularly PS and DM. (See table.) **Conclusion:** Temporary endoscopic gastric electrical stimulation is feasible and reproducible in a large numbers of patients. Temporary endoscopic gastric electrical stimulation, combined other emerging technologies, gives gastroenterologists the ability to screen patients implantation before implantation of permanent devices.

Category	B	T	B	T	B	T	B	T
MEASURE	N	N	V	V	TSS	TSS	4 H GET	4 H GET
ALL	3.4	0.9	2.5	0.6	15.2	5.1	20.7	20.6
DM	3.2	0.7	2.6	0.3	14.3	3.2	23.4	20.5
ID	3.2	0.2	2.2	0.6	14.7	4.7	12.9	13.1
PS	3.2	0.2	2.6	0.2	14.1	2.1	28.3	15.8
P VALUE		<0.0001		<.0.0001		<.0.0001		1/3 <0.05

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How Common in Rapid Gastric Emptying in Gastroparesis?

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Introduction: Rapid gastric emptying has been described not only in patients with nausea and vomiting and gastroparesis but also in obesity and dyspepsia. We investigated the prevalence of rapid gastric

emptying in a large group of gastroparetic patients compared to normal controls. **Patients:** From a pool 345 consecutive patients (67 males, and 278 females with a mean age of 42 years) with the symptoms of gastroparesis, we reviewed the results of standardized solid gastric emptying tests and compared the results to a groups of normal controls as previously described (AJG 95: 1456-1462). Measurable gastric emptying could be determined for gastric emptying in 265 patients; 212 patients had complete 1, 2 and 4 hour emptying, with diagnosis: 117 patients Idiopathic etiology, 62 73 patients with Diabetes Mellitus, and 27 Post Surgical disorders. **Methods:** Gastric emptying was compared by % remaining at 1 hour (<37%) for rapid and/or at 4 hours (>10%) for delayed emptying, using normal control data and techniques previously described (AGJ2004; 99:S45 and NGM 2005; 17: 470). **Results:** 144 (68 %) patients had delayed emptying by 4 hour% > 10%; 21 (9 %) patients had rapid emptying by the 1 hour value alone; and 47 (22 %) had neither, classified as normal. There were only small differences between the percent of each category by diagnosis. **Conclusions:** sssssRapid gastric emptying of a solid meal is not uncommon in patients with the symptoms of gastroparesis. Using these parameters to determine rapid gastric emptying awaits prospective use in other patients, including therapies for dyspepsia and obesity. Standardized measures for rapid gastric emptying may be useful in evaluating therapeutic outcomes for diverse disorders.

	Delayed		Normal		Rapid		Total
ID	74	35%	43	20%	10	5%	127
DM	43	20%	19	9%	5	2%	67
PS	27	13%	11	5%	6	3%	44
Total	144	68%	73	34%	21	10%	

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Pilot studies of the efficacy of gastric electrical stimulation for gastroparesis-US/European comparison

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For ASSESS (Associated Southern Sites of Electrical Stimulation Studies)*[1]*University of Mississippi, Jackson, MS,* [2]*University of Arkansas Medical Sciences, Little Rock, AR,* [3]*Medical University of South Carolina, Charleston, SC,* [4]*Univeristy of Tenneesee, Memphis, TN.*EDGES (European Data of Gastric Electrical Stimulation) ** Sahlgrenska University Hospital, Göteborg, Sweden, ***Gartnavel General Hospital, Glasgow, Scotland, *****Centre Hospitalier Universitaire, Rouen, France, combined as EXACTS (European across American Comparative Trial of Stimulation)*

Introduction: Gastric Electrical Stimulation (GES) is an accepted therapy for drug refractory gastroparesis. However, reports of efficacy have varied between centers. We aimed to compare results from several centers for the efficacy of GES by standardizing data between centers. **Patients:** Consecutive patients with the symptoms of gastroparesis were evaluated at each center located in Western Europe (3 centers) and Southern US (4 centers). **Methods:** Consecutive patients were recorded by demographics (age, sex, age), underlying diagnosis (ID=, DM = D, PS = P), % of centers where GET criteria were used, months (Mos) since implant, percentage change in symptoms (baseline to latest for Vomiting =Vom and GI Total Symptom Score =TSS) and the results summarized below. **Conclusion:** This is the first non-formal trial comparison of the use of GES for patients with the Symptoms of Gastroparesis. The specific localities studied (Both US & Europe) reveal similarities and difference between centers. Ongoing prospective comparisons of outcome data are feasible and may be warranted with the continued clinical use of GES.

Locat	No	F, M;yr	DX:I,D,P	GET	Mos	Vom	TSS
EUR	39	24,15;39	9,25, 5	100%	33	76.0	48.0
US	304	246,58;42	184,80,40	75%	49	46.0	40.0
ALL	343	270,73;41	193,105,45	88%	41	61.0	44.4

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A pilot multi-center comparison of the efficacy of gastric electrical stimulation for gastroparesis

Associated Southern Sites of Electrical Stimulation Studies, USA (ASSESS)

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Introduction: Gastric Electrical Stimulation (GES) is an accepted therapy for drug refractory gastroparesis. However, reports of efficacy have varied between centers. We aimed to compare results from several centers for the efficacy of GES in 4 Southern US sites. **Patients:** A total of patients with the symptoms of gastroparesis were evaluated at each center located in the USA. **Methods:** Consecutive patients were recorded by demographics (age, sex, age), underlying diagnosis (ID=I, DM=D, PS=P), whether GET criteria were used, months since implant, percentage change in vomiting and total GI symptoms (baseline to latest, as mean and median (Mdn) were recorded and summarized in the table below. **Conclusion:** This is the first non-formal trial comparison of the use of GES for patients with the symptoms of Gastroparesis. The specific localities studied (US) reveal both similarities and difference between centers. Ongoing prospective comparisons of outcome data are feasible and warranted with continued clinical use of GES.

Ctr	No	F,M;yrs	DX:I,D,P	GE	Mo	V(Mdn)	TSS(Mdn)
A	47	40,7;39	28,11,8	y	80	39 (38)	36 (38)
B	29	19,10;45	25,3,1	y	60	38 (67)	36 (28)
C	186	49,37;43	102,53,31	n	36	60 (100)	47 (53)
D	42	38,4;42	29,13,0	y	19	48 (75)	40 (100)
All	304	246,58;42	184,80,40	75%	49	46 (51)	40 (55)

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Multi-center comparison of the efficacy of gastric electrical stimulation for gastroparesis—a pilot study European data of gastric electrical stimulation (EDGES)

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EDGES

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Introduction: Gastric Electrical Stimulation (GES) is an accepted therapy for drug refractory gastroparesis. However, reports of efficacy have varied between centers. We aimed to compare results from several centers for the efficacy of GES by standardizing data between centers. **Patients:** Consecutive patients with the symptoms of gastroparesis were evaluated at each center located in Western Europe. **Methods:** Consecutive patients were recorded by demographics (age, sex, age), underlying diagnosis (ID, DM PS), whether GET criteria were used, months since implant, percentage change in symptoms (baseline to latest) and percentage of patients explanted were recorded and the results are summarized in the table below: **Conclusion:** sssThis pilot report is the first non-formal trial comparison of the use of GES for patients with the Symptoms of Gastroparesis. The specific localities studied (Western Europe) reveal both similarities and difference between centers. Ongoing prospective comparisons of outcome data are feasible and may be warranted with the continued clinical use of GES.

Cntr	No pts	F,M;Yrs	DX:I,D,P	GET Crt	Mos	V%,(Mdn)	TSS,(Mdn)
A	20	11,9; 47	7,8,5	y	37	95 (100)	48 (54)
B	7	5,2; 30	2,5,0	y		48 (50)	
C	12	8,4;37	0,12,0	y	28	85	
D							
ALL	39	24,15;39	9,25,5	100%	33	76	48

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Plasma ghrelin level is fluctuating during a fasted state and its peak is associated with gastric phase III-like contractions in conscious rats

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Background: While it is well known that the peak of plasma motilin levels is associated with gastric phase III contractions in humans and dogs, motilin receptors have not been found and administration of motilin failed to induce any phase III-like contractions in rats. Ghrelin is an orexigenic peptide originating from the stomach. Peripheral administration of ghrelin induces gastric phase III-like contractions in rats (J Physiol 550, 227–40, 2003). In a fed state, ghrelin accelerates gastric emptying (Scand J Gastroenterol 39, 1209-14, 2004) and changes contractile pattern from fed to fasted pattern in rats (J Physiol 550, 227–240, 2003). However, it still remains unclear whether plasma level of ghrelin is associated with gastric phase III-like contractions in rats. We studied the correlation between the gastric motility and plasma ghrelin level during a fasted state in conscious rats. **Methods:** In male SD rats, a strain gauge transducer was implanted on the antrum and intravenous catheter was inserted into the jugular vein. After a 24-hour fasting, gastric contractions were monitored before and after the administration of acyl ghrelin (0.2 nmol/kg/min for 5 min, i.v.). To investigate whether endogenous ghrelin is involved in mediating spontaneous phase III-like contractions, a growth hormone secretagogue receptor (GHS-R) antagonist (1 pmol/kg, i.v.) was administered. Motility index (MI) and frequency of gastric phase III-like contractions were compared before and after the administration of a GHS-R antagonist. Blood was obtained from the jugular vein every 8 min for 4-6 times and plasma level of acyl ghrelin was measured using RIA kit (Linco, MO). **Results:** Gastric phase III-like contractions were augmented following the ghrelin infusion. A GHS-R antagonist significantly attenuated MI of phase III-like contractions to 40.6 ± 7.2% (n = 4), compared to saline-controls (95.9 ± 6.9%, n = 3, $p < 0.01$). Frequency of phase III-like contractions was also significantly attenuated by a GHS-R antagonist (from 3.25±0.5 to 1.25 ± 0.5 times/30min; $p<0.01$). Plasma level of ghrelin was fluctuated between 175 pg/ml and 64 pg/ml. Just before the initiation of gastric phase III-like contractions, plasma ghrelin level reached at its peak then returned to the basal level within 8 minutes. **Conclusion:** A GHS-R antagonist significantly attenuated spontaneous gastric phase III-like contractions in conscious rats. Plasma ghrelin level was fluctuating and its peak was associated with gastric phase III-like contractions. These results strongly suggest that endogenous ghrelin regulates gastric phase III-like contractions in rats.

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Long term changes in diet contents modify fundic compliance in pigs

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Fundic compliance is regulated through a vago-vagal reflex originating mainly from the duodenum (Kellow, Delvaux et al. 1999). Nutrient specific increase in compliance is observed immediately after a duodenal infusion of lipids (Cherbut 2003). Long-term changes (2 weeks) in diet contents inhibit the pyloric motor response induced by duodenal lipids suggesting that lipid sensitivity could be partly modulated by the diet (Boyd, O'Donovan et al. 2003). The aim of our study was to evaluate the hypothesis that fundic compliance could also be modulated by long term changes in diet contents.

Fundic compliance was measured using a pneumatic barostat on 12 conscious pigs (40 ± 2 kg) fitted with a permanent access to gastric and intestinal lumen. The animals were divided into 3 groups depending on the nature of their diet. Three diets were given depending of the group: normal (N), high fat (40 % lipids, HF) and high glucides (68 % glucides, HG). The energy density of each of these diets was identical (4.95 $kcal.g^{-1}$) to cancel caloric dependant effect. These diets were ingested equally in each group for at least 2 weeks. Then compliance was measured in the fasted state before and after duodenal intralipid (Kabivitrum, 1 $ml.min^{-1}$). Compliance ($ml.mmHg^{-1}$) was evaluated from the volume/pressure curve obtained during step inflation (2 mmHg step until the pressure reaches 24 mmHg, 90 sec step) (Whitehead et al. 1997).

There was no change in fundic compliance between groups without intralipid (132 ± 32.3, 123 ± 46.6, 117 ± 20.6 $ml.mmHg^{-1}$ for N, HF and HG groups respectively, $p>0.05$). Intralipid increased, as expected, fundic compliance for N and HG groups (183 ± 41.6 and 148 ± 24.8 $ml.mmHg^{-1}$ for N and HG groups respectively, $p<0.05$). On the contrary, fundic compliance was decreased for HF group only after intralipid (86 ± 39.3 $ml.mmHg^{-1}$, $p<0.01$).

Long-term ingestion of high fat diet changes fundic compliance sensitivity to intralipid infusion. Whereas, duodenal lipids infusion increases compliance for low lipid diets, the opposite was observed with high fat diet. This effect is not caloric dependant. Furthermore, the diet history-dependant intralipid effect suggests that the duodenal mucosal receptors sensitivity to lipids is profoundly altered. Since in humans pharmacologically increased compliance is associated with reduced food intake (Schirra et al. 2002), the reduction in gastric compliance observed after nutrient stimulation in high fat diet group suggests that long-term ingestion of this diet is prone to inhibit gastric satiety signals.

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Biomagnetic detection of gastric slow wave changes evoked by glucagon

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Introduction: Several previous studies have examined the effect of hyperglycemia on the gastric slow wave. Bradygastria, tachygastria and arrhythmia have all been observed in serosal electrode recordings, but cutaneous electrodes appear to record predominantly tachygastria and arrhythmia. **Methods:** We used a multichannel Superconducting QUantum Interference Device (SQUID) magnetometer to measure the magnetogastrogram (MGG) from pigs (N=7) before and after intravenous injection of glucagon (20 mcg/kg) with an additional dose 20 minutes past the first dose. A magnetometer channel situated near the epigastric region was selected for analysis of the frequency content of the biomagnetic signature of the slow wave. We determined the dominant frequency (DF) of the MGG during baseline and at six time points (3, 10, 15, 25, 37 and 50 minutes) past the initial glucagon injection. At these same intervals, we determined the percentage of power distributed (PPD) in the MGG signal in bradygastric (1-3 cpm), normogastric (3-6 cpm) and tachygastric (6-9 cpm) ranges. **Results:** The gastric slow wave was observed in all magnetic recordings and confirmed with serosal electrodes. Immediately after injection of glucagon (3 min. post-injection), only moderate changes were observed in MGG recordings, and there was no statistically significant difference between DF (3.7 ± 0.3 cpm baseline vs. 3.1 ± 0.4 cpm at t=3 min) or PPD (15.6 ± 2.8%/74.5 ± 3.1%/9.9 ± 2.6% brady/normo/tachy at baseline vs. 16.0 ± 3.9%/73.2 ± 3.9%/10.8 ± 2.6% at t=3 min). However, ten minutes after glucagon was injected, we noted significant decreases in DF to 2.7 ± 0.5 cpm ($p < 0.05$) and in normogastric PPD to 59.2 ± 4.3% ($p < 0.05$), and a significant increase in bradygastric PPD to 31.2 ± 3.2%. However, the tachygastric PPD was not statistically different from the baseline value (9.6 ± 3.0%). After 15 minutes post-injection, the slow wave parameters returned toward pre-glucagon values with , but when the second dose was administered after 20 minutes, both DF and PPD values were again significantly different from baseline ($p<0.01$, brady PPD & DF; $p<0.05$, normo PPD). **Conclusions:** These results suggest that the magnetogastrogram reflects changes in the gastric slow wave in response to glucagon. Interestingly, we did not observe a significant increase in tachygastric frequencies. We believe that this likely reflects the excellent DC response of SQUID magnetometers, whereas cutaneous electrodes are more susceptible to low frequency noise caused by junction potentials at electrode interfaces.

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Effects of lipase inhibition on gastric emptying and alcohol absorption in healthy subjects

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Alcohol absorption is dependent on the rate of gastric emptying (GE). As the slowing of GE by fat is dependent on lipolysis, orlistat may

increase the rise in blood alcohol when alcohol is consumed with, or after, fat. The aims of this study were to evaluate the effects of orlistat on GE and plasma alcohol after an alcohol-containing drink following a fat 'preload', in healthy subjects. Ten healthy males (mean age 29.5 ± 3.7 years), consumed 120ml of cream with or without 120mg orlistat, 30min before an alcohol-containing drink labeled with 20MBq ^{99m}Tc-sulfur-colloid on two days while seated in front of a gamma camera. GE, plasma alcohol and blood glucose were measured for 240min. GE was slightly faster (P<0.05) with orlistat compared to control (Figure a). The initial rise in plasma alcohol was slightly higher (P<0.05) i.e. at 15 min with orlistat (0.034 ± 0.006g/100ml) vs control (0.029 ± 0.005g/100ml), but there was no difference after this time (Figure b). The increase in blood glucose from baseline was greater (P=0.05) with orlistat eg. at 15min (1.07 ± 0.2mmol/L) vs control (0.75 ± 0.2mmol/L). The rise in blood glucose and plasma alcohol were related (eg at 15min r = 0.49, P = 0.03).

In conclusion, lipase inhibition accelerates GE of an alcohol-containing drink following a fat ''preload'' with a minor increase in the initial rise in plasma alcohol.

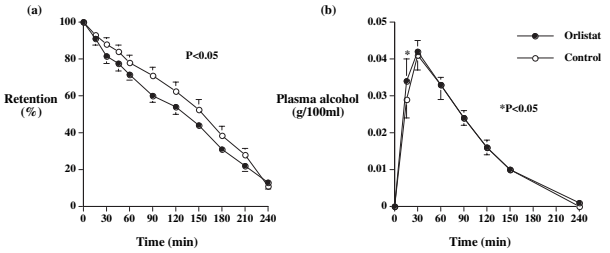

Figure 1 Effects of orlistat on (a) gastric emptying and (b) plasma alcohol levels, following ingestion of a fat 'preload' and an alcohol-containing drink. Data are mean values ± SEM.

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Regional gastric slow wave propagation and coupling measured by endoscopy-directed multichannel gastric mucosal recording in healthy humans: effects of acute hyperglycemia

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Background: Current methods to measure in vivo human gastric slow waves quantify rhythm and power but cannot describe coupling between adjacent regions or propagation properties. We previously developed a bipolar mucosal probe to measure slow waves at a single site that, when directed to different areas, characterized region-specific rhythm changes and gave indirect evidence of uncoupling with dysrhythmic stimuli. **Aims:** In this study, we used an endoscopy-directed multichannel probe to quantify regional slow wave rhythm, power, propagation, and coupling during control conditions vs. hyperglycemia. We hypothesized that hyperglycemia preferentially disrupts distal rhythm, propagation, and coupling. **Methods:** A catheter with 3 bipolar electrodes was affixed 2, 6, and 10.5 cm from the pylorus with hemoclips during upper endoscopy with midazolam in 6 healthy humans. After 30 min equilibration, a 1 hr basal recording was done. Recording continued with hyperglycemic clamping to 250 mg/dl x 1 hr. **Results:** In control recordings, slow waves were continuously detected with similar rates in proximal (2.91±0.09 cycles/min[cpm]) and distal leads (2.87±0.11 cpm). Abnormal rates (<2.4 or >3.6 cpm) were seen 6.1±1.3% of the time (tachygastria 2.2±0.8%, bradygastria 3.9±0.5%). Distally power was 2.9±0.6 fold greater and temporal variability of power was 2.6±1.3 fold greater than seen proximally (both P<0.03). Antegrade coupling between adjacent sites was 74±8% with <50% coupling in 3±2% and retrograde coupling in 4±2% of 5 min segments. Propagation velocities were 1.3±0.4 cm/sec proximally and 1.2±0.6 cm/sec distally (P=NS). Hyperglycemia increased distal (3.43±0.12 cpm, P<0.02) but not proximal (3.06±0.12 cpm, P=0.39) rates and increased times in abnormal frequency (26.4±3.4%, P<0.002). A trend to tachygastria distally (26.8±8.3%) vs. proximally (9.0±5.1%, P=0.13) was noted. Hyperglycemia did not affect power (P=0.74) or power variability (P=0.94) but reduced coupling to 51±6% proximally and 41±1% distally (both P<0.02). % of time with <50% coupling increased proximally (44±11%) and distally (74±6%)(both P<0.02) and retrograde coupling increased to 30±7% (P<0.05). **Conclusions:** sEndoscopy-directed multichannel mucosal recording uniquely quantifies gastric slow wave frequency, power, propagation, and coupling in humans. Hyperglycemia elicits preferential distal tachygastria, loss of slow wave coupling, and retrograde conduction. This novel method shows promise as a means of characterizing in vivo myoelectric conduction disturbances in disorders such as gastroparesis and functional dyspepsia.

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A quantitative model of gastric smooth muscle cellular activation

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A physiologically realistic quantitative description of the electrical behaviour of a gastric smooth muscle cell is presented. The model describes the response of a smooth muscle cell when activated by an electrical stimulus coming from the network of Interstitial Cells of Cajal (ICC) and is mediated by the activation of different ion channels species in the plasma membrane. The classical Hodgkin-Huxley approach of modelling the cell membrane has been employed. The conductances that are believed to substantially contribute to the membrane potential fluctuations during slow wave activity have been described in the model. These include an L-type and a low voltage activated Ca^{2+} channels; A-type, delayed rectifier and large conductance Ca^{2+}-activated K^+ conductances; a Na^+ channel and a non specific cationic conductance. All the parameters that characterise the ion channels kinetics have been taken, whenever possible, from patch clamp experimental data on freshly dispersed gastric smooth muscle cells found in literature. A phenomenological description of intracellular Ca^{2+} dynamics has also been included because of its primary importance in regulating a number of cellular processes. The stimulus coming from the ICC network has been characterized according to published traces of ICC gastric slow waves. In terms of shape, duration and amplitude, the resulting simulated slow waves are in good agreement with experimental recordings from mammalian gastric smooth muscle. Also the predicted raise in intracellular Ca^{2+} during the slow wave is in good agreement with experimental measurements. To further validate the model, the action of some known potassium channel blockers has been simulated by accordingly varying the respective K^+ conductance(s): presence of flecainide in concentration near its IC_{50} (30 μM) was simulated by halving the A-type K^+ conductance and the presence of 4-AP (5mM) was replicated by setting both A-type and delayed rectifier conductances to zero; good correspondence between simulations and experimental slow wave recordings in such altered conditions has been found. This model has been designed to be suitable for incorporation into large scale simulations for the study of the spatial propagation of gastric slow waves.

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Characterization of gastroduodenal motility in healthy subjects and patients with gastropareis using an ambulatory capsule

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Introduction: Do the patients with gastroparesis, in addition to delayed gastric emptying, have gastroduodenal motility abnormalities that can be identified and described using capsule technology? **Aim:** To compare pressure patterns between healthy subjects and patients with gastroparesis. **Methods:** In a multicenter study, healthy subjects and patients with gastroparesis swallowed the SmartPill (SP) capsule after an overnight fast together with a standardized meal (120 g Eggbeaters, 2 pieces of bread with jam; 255 kcal, 2% fat) and 120 cc water. The rapid and persistent luminal pH rise (above 4 and at least 3 unit rise from baseline gastric pH) marked the emptying of the ingested SmartPill

from the stomach into the duodenum. The frequency and amplitude of contractions recorded by the capsule were analyzed in 20 minutes intervals from 60 min before gastric emptying (GE) to 60 min after. These parameters for patients and controls were compared for each time interval by two-sample unequal variance t test.**Results:** 66 healthy subjects (42M/24F) and 38 patients, 16 diabetic/22 idiopathic (8M/30F) were studied. When comparing patients to the control group, for (–60 to –40 min) and (-40 to –20 min) before gastric emptying, patients had significant lower frequency of contractions (0.6 /min, [95% CI 0.3-0.9] and 0.8 /min, [95% CI 0.4-1.2] respectively) compared to healthy subjects (1.2 /min, [95% CI 1.0-1.4], p< 0.002 and 1.4 /min, [95%CI 1.2-1.6], p <0.009 respectively). When comparing healthy subjects (N) with subtypes of gastroparesis, a highly significant difference in the frequency of contractions is seen for the diabetic group (D) earlier (–60 to –40 min) before the emptying (frequency for N = 1.2/min, for D = 0.45 / min, p<0.0001). The differences in frequency for the idiopathic (I) group had stronger significance in a later period (–40 to –20 min) (frequency for N = 1.39 /min, for I= 0.70 /min, p <0.002), suggesting variation in the motility abnormalities that potentially differentiate the 2 gastroparetic groups. There was no difference in the mean amplitude of contractions for patients and controls. **Conclusion:** An ambulatory capsule SmartPill measuring contractile patterns can demonstrate differences in upper GI motility between healthy volunteers and subtypes of patients with gastroparesis. Research funded by SmartPill Corporation.

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Gastric electrical stimulation in medically refractory nausea and vomiting

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Introduction: High frequency gastric electrical stimulation (HF-GES) is a new therapeutic option to improve refractory nausea and vomiting, in gastroparetic patients. However, its effects on gastric emptying are inconstant and limited. Therefore, we have hypothesized that HF-GES could be also effective in patients suffering from refractory vomiting and nausea with normal gastric emptying and we have compared the symptomatic efficacy of HF-GES between patients with delayed and normal gastric emptying. **Patients and Method:** Fifteen patients with chronic, severe and medically resistant nausea and vomiting were included in the study. Gastric emptying was delayed in 8 patients (Group 1) and normal in 7 patients (Group 2). At inclusion and at 6 months after the start of the stimulation, symptoms (nausea and vomiting, bloating, regurgitations, abdominal pain and appetite) and quality of life were prospectively evaluated using the GIQLI score while gastric emptying was assessed by scintigraphy and/or octanoic acid breath test. **Results:** Age, gender, symptoms and quality of life were not different at baseline between the 2 groups. At 6 months, GIQLI and nausea/vomiting scores had significantly improved in both groups. Other symptoms (bloating, regurgitations, abdominal pain and appetite) had improved at 6 months in Group 1 but not in Group 2. Six months after the start of stimulation, gastric emptying was normal in 4/8 Group 1 patients and 5/7 Group 2 patients but was not significantly different from that calculated before the implantation of the stimulator. **Conclusion:** Our results suggest that HF-GES is an effective therapy for treating chronic, severe vomiting and nausea whatever gastric emptying is delayed or not.

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Selective intragastric pH profile impairment in diabetic vs. idiopathic gastroparesis: relation to degree of gastric stasis

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Background: The acid milieu is poorly understood in gastroparesis of different etiologies and varying degrees of stasis. Clinical evidence suggests diabetic and idiopathic gastroparesis have distinct pathophysiology. Diabetics with gastroparesis may have bacterial overgrowth, which also occurs with achlorhydria, while dyspeptics with delayed emptying may respond to acid suppressants suggesting differences in gastric acidity in the 2 disorders. Recently, a capsule was developed which constantly measures gut pH (SmartPill Corp.). **Hypotheses:** We hypothesized (i) gastric pH profiles show differential impairment in diabetic vs. idiopathic gastroparesis and (ii) abnormal pH profiles relate to severity of stasis. **Methods:** 64 healthy controls and 44 gastroparesis patients (20 diabetic, 24 idiopathic) from 7 centers were analyzed. Acid suppressants were stopped for 1 wk. After capsule ingestion, subjects ate meals (120 g Egg Beater, bread, jam, and 120 ml water; 255 kcal) with 1 mCi ^{99}Tc-sulfur colloid. pH was measured every 5 sec; scintiscans were taken every 30 min x 4-6 h. **Results:** Basal pH was higher in diabetics (3.64±0.41) vs. controls (1.90 ± 0.18) and idiopathics (2.41±0.42)(both P<0.05). Meal-evoked peak pH was greater in diabetics (4.98±0.32) than idiopathics (3.89±0.39)(P=0.04) and intermediate in controls (4.48±0.14). This declined to pH nadirs that were higher in diabetics (1.50±0.23) than controls (0.58±0.11)(P<0.001) and idiopathics (0.93±0.31). Summed fed reacidification quantified by area under the pH curve (AUC)(pH x hr) was highest in diabetics (4.10±0.51) showing less acidity than controls (2.93±0.20) and idiopathics (2.61±0.40)(both P<0.02). 90% emptying times (T90) were similar in diabetics (249±15 min) and idiopathics (249±16 min) but higher than controls (162±5 min). Versus controls, those with profound stasis (T90>300 min) had higher basal (3.91±0.55), peak (5.30±0.50), and nadir pH (2.23±0.42), and AUC (4.63±0.86)(all P<0.05). pH and AUC in mild gastroparesis (T90<300 min) were similar to control. **Conclusions:** Diabetics with gastroparesis exhibit lower gastric acidity than controls (?vagal neuropathy) while idiopathic gastroparetics show nearly normal profiles. Patients with severe stasis exhibit higher pH regardless of etiology. This study characterizes physiologic differences in diabetic and idiopathic gastroparesis of varying severity which may relate to consequences of altered gastric acid including dyspepsia and bacterial overgrowth.

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Circulating ghrelin level is decreased according to the extent of atrophic gastritis

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Backgroud: Ghrelin, a novel peptide recently identified in the stomach of the rodents and humans is known as a potent stimulator of growth hormone release and food intake. Ghrelin is also known to stimulate the mobility of the stomach and the acid secretion of the stomach. Although the stomach is the main source of circulating ghrelin, the regulation of gastric ghrelin secretion is poorly understood. In this study, we undertook to clarify that *Helicobacter pylori* (HP) infection and the atrophic change of gastric mucosa affect plasma ghrelin concentration. **Method:** Plasma ghrelin (deacylated-ghrelin and acylated-ghrelin) concentration in patients with peptic ulcer or atrophic gastritis and healthy subjects (168 subjects) were measured by enzyme immunoassay in Mitsubishi Kagaku Iatron, INC. The extent of atrophic change of gastric mucosa was assessd endoscopically. *Helicobacter pylori* (Hp) infection was determined by the assay of anti-Hp antibody. **Results:** Plasma deacylated-ghrelin concentration of Hp negative group was significantly higher than that of Hp positive group (121.0 ± 12.9 fmol/ml versus 77.0 ± 6.2 fmol/ml). Plasma acyl-ghrelin concentration in patients with non- or mild atrophic gastritis was significantly higher than that in patients with moderate and severe atrophic gastritis (9.3 ± 1.1 fmol/ml versus 3.3 ± 0.6 fmol/ml or 4.8 ± 0.5 fmol/ml). Plasma deacyl-ghrelin concentration in patients with non-mild atrophic gastritis was also significantly higher than that in patients with severe atrophic gastritis (143.8 ± 16.6 fmol/ml versus 61.0 ± 4.9 fmol/ml). If the patients were separated into two groups by the presence or the absence of HP infection, plasma ghrerin concentrations were also decreased according to the extent of atrophic gastritis in the two groups. There was no significant difference in plasma

ghrelin concentration in patients between the two groups with or without HP infection, if they have the same extent of atrophic change in stomachs.**Conclusion:** The results suggest that plasma ghrelin concentration is decreased according to the extent of atrophic change of gastric mucosa, indicating that the low level of plasma ghrelin in patient with HP infection is caused by the development of atrophic gastritis.

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Dietary free glutamate promotes gastric emptying of the protein-rich liquid meal in adult humans

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Background and Aim: Free glutamate activates the taste receptors in the oral cavity and excites taste nerves to elicit a unique taste 'umami'. Ingestion of glutamate enhances the secretion of saliva, gastric juice and pancreatic juice. The series of evidence in nutrition have shown that glutamate is a signal of protein intake. Recently two types of G-protein coupled receptor, CaSR and mGluR1, that sense amino acids were found in the gastric mucosa, suggesting free glutamate in the stomach lumen modulate gastric function. In this study we examined the effect of dietary free glutamate on human gastric emptying of 2 types of liquid meals, protein-rich meal and carbohydrate meal. **Methods:** Two types of control liquid test meal (400 kcal/400 ml) labeled with 100 mg of 13C sodium acetate were used. Protein-rich meal consisted of 12.5% dextrin and 12.5% casein-calcium; Carbohydrate meal contained 25% dextrin. Monosodium L-glutamate (0.5% w/v, Ajinomoto Co., Inc., Japan) was added to a control liquid test meal. To investigate whether the presence of nutrients influences the effect of glutamate, 400ml of water was also tested. After taking a test meal or water, continuous 13C breath tests were performed. **Results:** Protein-rich meal (n = 10): glutamate significantly promoted gastric emptying rate. The gastric emptying coefficient (GEC) defined by Ghoos et al (glutamate +/−) was 3.05 ± 0.24 / 2.69±0.28 (mean ± S.D., $p<0.01$, multiple ANOVA). The t 1/2 (glutamate +/−) was 153.0 ± 34.6 /212.7 ± 102.6 minutes (mean±S.D., $p<0.05$). The cummulative %dose at 180-min was 59.2 ± 9.8 /45.8±14.4 (mean±S.D., $p<0.05$). (2) Carbohydrate meal (n = 9): glutamate had no significant effect on gastric emptying. (3) Water (n = 9): glutamate had no significant effect on gastric emptying. **Conclusions:** This report shows for the first time that dietary free glutamate promotes gastric emptying in humans. Glutamate facilitated the gastric emptying of a protein-rich meal without influence on that of water or carbohydrate meal. These data suggest that free glutamate is an important substance for efficient protein digestion and absorption. The reevaluation of the GI function from the standpoints of chemical sense will help to understand better the pathophysiology of dyspeptic symptoms.

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Secondary waves initiate gastric arrhythmias and reentry in the canine stomach in vivo

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The mechanisms that initiate spontaneous gastric arrhythmias are unknown but could be based on focal activation or reentrant propagation. The aim of this study was to elucidate this mechanism by analyzing the pattern of propagation during gastric arrhythmias in an *in vivo* canine model.

Eight fasted dogs were anesthetized and, after a median laparotomy, the ventral surface of the stomach was exposed *in situ*. A multi-electrode assembly of 240 electrodes (covering a 4 by 4 cm area with 2.5 mm inter-electrode distance) was positioned on the serosal surface of the stomach. After a 10-30 minute stabilization period, electrical activities were recorded for 5-15 minutes from all 240 electrodes simultaneously. The timings of slow waves at each electrode were determined off-line and slow wave propagation maps of the recorded area were constructed.

In the majority of cases, slow wave propagation was regular, homogeneous and uniform (4.8 ± 0.3 cycles/min). They propagated from corpus to antrum at increasing velocity and terminated in the pyloric region. Occasionally, secondary waves occurred in localized areas, 2-4 seconds after the upstroke of the regular slow wave, which occasionally initiated extra slow waves. When extra slow waves occurred, they were always (n = 22/22) initiated by a secondary wave, never by the regular slow waves themselves (n = 0/22). These extra slow waves had similar deflections as the preceding regular slow wave but occurred at much shorter intervals (4-6 seconds instead of the regular 12 seconds). These extra slow waves propagated in various directions and, occasionally (n = 12 in 22 extra slow waves), the extra slow waves conducted in a loop and re-entered previously excited areas.

In conclusion, spontaneous arrhythmias in the stomach appear to be caused by focal activities that initiate premature extra slow waves that in turn may degenerate into circus movement propagations. The fact that these combined focal/reentrant activities occur spontaneously in fasted anaesthetized animals seems to indicate that focal and reentrant activity in the stomach may be more common than previously recognized.

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Intra-esophageal infusion of hydrochloric acid increase the gastric mechanosensitivity in healthy subjects and patients with functional dyspepsia, and may increase compliance in healthy but not in dyspeptic patients

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Aim: To determine if the infusion of hydrochloric acid (HCl) into distal esophagus modulates the mechanosensitivity of proximal stomach in patients with functional dyspepsia (FD). **Methods:** In eleven patients with FD (five men, age: 37.7 ± 14.2, mean ± SD) and 12 healthy volunteer (ten men, age: 23.7 ± 1.5; presented data at 13th AMS), gastric barostat was used. Stepwise isobaric distensions of proximal stomach were performed and perception was measured before and after the intraesophageal infusion (5 ml/minutes for 20 minutes) of 0.1 N HCl or saline in a randomized, double-blind design. Statistics used paired Wilcoxon's test and ANOVA. **Results:** Intra-esophageal saline infusion had no significant effects on gastric mechanosensitivity. After esophageal HCl infusion, intragastric pressure at the maximum tolerable threshold was significantly decreased in healthy (16.3 ± 4.3 vs. 11.0 ± 2.8 mmHg, before and after infusion respectively, $p < 0.05$) and tend to decrease in dyspeptic patients (17.0 ± 5.2 vs. 13.7 ± 5.4 mmHg, $p = 0.09$), while pressures at thresholds for discomfort and first perception were tended to decrease in both group. VAS scores for fullness at 12 mmHg in healthy (3.4 ± 2.4 vs. 6.0 ± 2.9 mm, $p < 0.05$) and those at 6 mmHg and 8 mmHg in dyspeptic patients (0.9 ± 1.6 vs. 2.4 ± 1.9 mm; 0.9 ± 0.5 vs. 2.8 ± 1.6 mm, $p < 0.05$) were significantly increased after esophageal HCl infusion, while VAS scores for fullness at 8 mmHg in healthy and for nausea at 6 mmHg in both groups during gastric distension were tended to increase after esophageal HCl infusion. During stepwise isobaric gastric distension, esophageal HCl infusion was tended to increase compliance in healthy (171.7 ± 119.7 vs. 456.7 ± 333.3 ml, $p = 0.065$), but not in dyspeptic patients. **Conclusion:** Intraesophageal HCl infusion increases mechanosensitivity of proximal stomach in healthy subjects and FD patients. However it also may increase compliance of proximal stomach in healthy subjects, but not in FD. These finding suggest the possible role of central sensitization during esophageal acid reflux and possible defect of compensatory relaxation of proximal stomach in the pathophysiology of FD. Supported by 3rd Choongwae Grant of the Korean Society of Neurogastroenterology and Motility.

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Effects of gastric electrical stimulation (GES) on burn induced delayed gastric emptying

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Background and Aim: Severe burn injury is known to delay gastric emptying in both animals and humans. Delayed gastric emptying hinders early enteral feeding which is beneficial in treating burn patients. The aim of this study was to investigate if gastric electrical stimulation (GES) was capable of improving burn induced delayed gastric emptying. **Methods:** 18 rats were randomly divided into 3 groups: sham burn group (control group), burn group and burn with GES group. One pair of cardiac pacemaker wires was surgically implanted to gastric serosa 2.0 cm from the pylorus. Following a 3-day recovery, the rats received a sham or a 3rd degree scald burn under anesthesia. Proper fluid resuscitation and analgesia were given to all animals. Six hours after sham/burn, GES was performed continuously for 1 hr with a frequency of 5.5 pulses/min, amplitude of 5mA and a pulse width of 400ms. A test meal (1.5% methylcellulose meal) was mixed with phenol red (marker), and was given orally 30min after the initiation of GES. The rats were sacrificed 30 min after the meal. The stomach was harvested and the retention of phenol red in the stomach was calculated using a previously established method. **Results:** Severe burn impaired gastric emptying, compared with that of the control group, gastric emptying of burnt rat was significantly delayed (76.6 ± 3.7% vs. 45.4 ± 2.9%, P<0.001); and this burn-induced delayed gastric emptying was improved with GES (85.28 ± 2.89%, P<0.001). **Conclusion:** Severe burn injury delays gastric emptying in rats. Gastric electrical stimulation with long pulses normalizes the burn induced delayed gastric emptying. Further studies are needed to explore the therapeutic potential of GES in treating burn patients with delayed gastric emptying.

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SPECT for gastric volume assessment: a method with observer defined regions of interest

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Aim: SPECT imaging allows non-invasive measurement of gastric volume. In previous studies, the processing of the SPECT data involved global threshold algorithms that do not take into account the non-uniform distribution of radioactivity in the gastric wall. The purpose of this study was to develop a simple and robust scintigraphic method based on observer defined regions of interest to measure gastric volume. **Methods:** A phantom study was performed to standardize volume calculations from a sequence of SPECT derived cross sectional areas. Thereafter, twelve healthy volunteers were included in the study. At baseline, determination of gastric volume was based on SPECT imaging after intravenous injection of about 200 MBq ^{99m}Tc-pertechnetate. After ingestion of about 2 MBq ^{111}In-diethylenetriaminepentaacetic acid in a 600-ml liquid meal, dual-isotope technique with SPECT and planar imaging assessed gastric volume and gastric emptying. Repeated calculations derived from SPECT data from three of the volunteers were used to evaluate the reproducibility of the volume measurements. **Results:** The median volume of the stomach was 86 ml (range 62-130 ml) at baseline, 642 ml (536-748 ml) immediately after the meal, and 370 ml (221-481 ml) 1 h after the meal. The coefficient of variation for the calculations was 9%, 2%, and 4%, respectively. The median increase in gastric volume was 562 ml (501-628 ml) immediately after the meal and 294 ml (159-370 ml) after 1 h. Gastric retention of the meal was 68% (50-73%) after 0.5 h and 51% (39-57%) after 1 h. **Conclusion:** The present manual technique may be a reliable alternative to the automated methods for assessing gastric volume before and after meals. The liquid meal that was used in our study did not seem to cause an increase in gastric volume that differed from the volume of the meal.

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Gastric emptying and myoelectrical activity in patients with typical reflux symptoms

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Background: Very few studies have focused on the myoelectrical activity of stomach in Gastro Oesophageal Reflux Disease (GORD). There is evidence to suggest that delayed Gastric Emptying (GE) is demonstrable in a significant proportion of patients with GORD. Electrical Impedance Tomography (EIT) when used with a conductive meal can non-invasively assess gastric emptying and complements the Electrogastrography (EGG) in assessing myoelectrical activity

Our hypothesis was that gastric emptying and the electrical activity of stomach is abnormal in GORD and contributes to reflux of gastric contents into the oesophagus. **Aims:** To assess gastric emptying and myoelectrical activity in patients who experience typical symptoms of GORD. **Methods:** 28 consecutive patients with typical reflux symptoms lasting at least a year, characterised by heartburn (relieved by antacids exacerbated by stoppage of proton pump inhibitor) and regurgitation were recruited. GE was measured using EIT (DAS-01P APT system; University of Sheffield, UK) after administering a semisolid meal. Gastric myoelectrical activity was recorded for 24 hours using a portable EGG recorder. Patients were asked about the presence of dyspeptic symptoms, epigastric pain, postprandial bloating, belching, nausea, and vomiting. We also enquired about the presence of dysphagia, cough and dysphonia. **Results:** The gastric half emptying times estimated by EIT were delayed in 11 of the 28 patients analysed and the GE50 values were 137.45 ± 24.39 min in these patients. The dominant frequency of the stomach pacemaker electrical activity was abnormal in 15 of the 28 patients, all exhibiting significant periods of bradygastria on the 24 hour recordings. 9 of the 11 patients who had delayed gastric emptying on EIT also showed abnormal percentage of bradygastria in their total duration of recording. All the patients who showed delayed gastric emptying also complained of postprandial bloating and belching on a regular basis. **Conclusions:** Gastric emptying was delayed in 39% of patients with GORD and 53 % also had bradygastria. There was good correlation between dyspeptic symptoms and delayed gastric emptying.

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Slow wave frequency gradient in stomach detected by a Magnet-Tracking System

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Background: Slow wave frequency gradients have been described in the human small intestine both *in vivo* and *in vitro*. The Magnet Tracking System, a new non-invasive technique, can monitor the position of a magnet in the gastrointestinal tract and its linear or rotational displacements. Previous studies examining motor patterns in the GI tract, using a freely moving magnet, suggested a possible gradient of slow wave frequency within the stomach. **Aim:** To assess whether a gradient in gastric slow wave frequency exists in healthy volunteers. **Methods:** Six healthy subjects (4 F, mean age 34 yrs) participated in the study. A magnet (10x3 mm) was attached by a silk string (1.5 cm) between two distal sensors of a manometric catheter (4 solid state transducers, 5 cm apart). The catheter was passed transnasally and the gastric motor patterns were recorded after positioning the magnet at 45 and 50 cm from mouth (15 min at each position) and then at 55 cm for 100 min. Subsequently, the subjects were given a liquid meal (245 calories) and the motility was recorded for 15 more minutes. The magnet position and spontaneous movements were monitored using an external sensor array located over the abdomen and connected to a computer. Pressure changes were recorded using an ambulatory recording system. **Results:** All six subjects displayed higher slow wave frequency at 45 cm (3.28+0.61 cpm) than at 55 cm (2.75+0.39, p<0.01). All but one subjects showed a decreasing gradient in slow wave activity frequency between 45 cm and 55 cm. This subject had a

lower slow wave frequency at 50 cm than at 55 cm. An increase in the gastric slow wave frequency was detected in 5 subjects during postprandial period (2.97+0.45, p>0.05). Gastric contractions detected by manometry were always detected by Magnet Tracking System. However, not every magnet displacement/movement was reflected by pressure changes. **Conclusions:** There is a frequency gradient of gastric slow waves in healthy volunteers, with a higher frequency in the proximal stomach. An increase in gastric slow wave frequency detected post-prandially may reflect entrainment of the distal stomach due to distension or luminal stimulation.

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No association between changes in symptoms and gastric emptying in gastroparetic patients treated with gastric electrical stimulation

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The aim of this study was to investigate whether there is an association between gastric emptying rate and symptom improvement in gastroparetic patients treated with gastric electrical stimulation (GES). **Methods:** We retrospectively reviewed 63 gastroparetic patients (12M, 51F, mean age: 41 years, range: 21-66; 38 diabetic, 11 idiopathic and 14 postsurgical) who had documented delayed gastric emptying of a solid meal and received GES therapy for at least 1 year. Patient characteristics, total symptom score (TSS) derived from 7 upper gastrointestinal symptom sub-scores (0-4) and 4-hour standardized gastric emptying test (GET) were evaluated at baseline and at 1 year of GES. Data are presented as Mean (SE). **Results:** Of 63 patients, 14 had their GET normalized and 49 remained delayed GET at 1 year of GES (normal value at 2 hours <60% and at 4 hours <10% gastric retention). There was no difference in mean TSS reduction at 1 year between patients with normalized GE and those with delayed GE (61% vs. 59%). 9/14 (64%) patients with normalized GE and 31/50 (62%) with delayed GE at 1 year had a ≥50% reduction in TSS at 1 year (see Table below). Overall 33 patients did demonstrate decreased gastric retention while 30 patients had worsening of their GET. There were no differences in symptom improvement between the patients with a better GET and those with a worse GET at 1 year (see Table below). **Conclusions:** There is no association between changes in symptoms and gastric emptying in gastroparetic patients treated with GES, suggesting that the effect of GES is due to factors beyond gastric motility.

	Vomiting (0-4)		Nausea (0-4)		TSS (0-28)	
Mean (SE)	BL	1 year	BL	1 year	BL	1 year
Pts with normal GE at 1y (14)	2.9 (0.4)	0.8 (0.2)*	3.9 (0.1)	1.4 (0.4)*	22 (1.1)	9.4 (1.6)*
Pts with delayed GE at 1y (49)	2.8 (0.2)	1.1 (0.2)*	3.4 (0.1)	1.5 (0.2)*	19.3 (0.7)	8.6 (1.0)*
Pts with improved GE (n=33)	2.9 (0.3)	1.2 (0.2)*	3.7 (0.1)	1.6 (0.2)*	19.6 (0.7)	9.4 (1.1)*
Pts with worse GE (n=30)	2.9 (0.3)	1.0 (0.2)*	3.5 (0.1)	1.5 (0.2)*	19.8 (1.0)	8.5 (1.3)*

*P<0.01 compared to baseline; TSS, total symptom score, GE, gastric emptying, BL, baseline.

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Relationship between intra-gastric meal distribution and delayed gastric emptying in critical illness

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Slow gastric emptying and subsequent intolerance of gastric feeds occur commonly in critically ill patients. Both proximal and distal gastric dysmotility as well as disruption of the coordination between the 2 regions have been reported. Currently, there are limited data on intra-gastric meal distribution or the contribution of each gastric region to delayed gastric in these patients. **Aims:** To determine the impact of critical illness on intra-gastric meal distribution and its relationship to delayed gastric emptying. **Methods:** Gastric emptying and intra-gastric distribution of 100ml of Ensure, labelled with 20MB Tc^{99}, were assessed prospectively in 24 mechanically ventilated, critically ill patients (11M; 63 ± 4 yrs; APACHE II 22 ± 2) and 20 healthy volunteers. Scintigraphic measurements were performed over 4h to determine (i) gastric emptying half time ($t_{1/2}$); (ii) overall % meal retention (at 60, 120, 180 and 240min), and (iii) meal retention in proximal and distal stomach. Delayed gastric emptying was defined as $t_{1/2}$ >60min. **Results:** Delayed gastric emptying occurred in 54% (13/24) critically ill patients. In patients, both median $t_{1/2}$ (61 (35-228) vs. 34 (28-51)min; P=0.02) and gastric retention after 120 min (P<0.01) were significantly greater than in healthy subjects. In both groups, more than 2/3 of the meal was initially distributed in the proximal stomach. In patients, a significantly greater proportion of meal was retained proximally after 120 min (P<0.01). Proximal retention was greater after 60 min in critically ill patients with delayed gastric emptying (p<0.05), compared with patients with normal gastric emptying or healthy subjects. **Conclusions:** In critical illness, slow gastric emptying is associated with increased proximal gastric meal retention, suggesting disturbed proximal gastric motility and/or coordination between the 2 gastric regions is important in the pathogenesis of gastroparesis in these patients.

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Disruption to the functional association between proximal and distal gastric motor activity in critically ill patients

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Normal gastric emptying is complex and involves coordination of proximal and distal gastric motor activity in response to small intestinal nutrient feedback. Fundic waves (FWs) may play a role in transferring gastric contents more distally for antral trituration and emptying. Slow gastric emptying and subsequent intolerance of gastric feeds occur in 50% of critically ill patients. Both proximal and distal gastric motility are abnormal in these patients, but the effect of critical illness on the coordination between these regions is unknown.
Aim: To examine the effects of critical illness on functional association between proximal and distal gastric motility, during fasting and nutrient stimulation. **Methods:** Concurrent proximal (barostat) and distal (perfused multi-lumen manometry) gastric motility were recorded in 10 critically ill patients and 10 healthy volunteers, during fasting, and two 60-min duodenal infusions of Ensure (1kcal/min and 2kcal/min) administered in random order separated by a 2-h ''washout'' period. Proximal gastric volume (PGV), fundic wave (FW) activity, antral waves and the association between these variables were assessed. Data are mean ± SEM unless stated otherwise. **Results:** During fasting, PGVs were similar (Pts: 206±47mL vs. healthy: 196±274mL, P=0.7) but critically ill patients had fewer FWs and propagated antral waves (PAWs) than healthy subjects. During duodenal nutrient infusion, there was less proximal gastric relaxation (at 10^{th} min: 45±26 vs. 196±29mL; P<0.001) and fewer FWs (4.3±1.2 vs. 8.8±1.2 wave/10min, P=0.003) and PAWs (0.4±0.1 vs. 4.5±1.8 wave/10min, P<0.001) in patients than in healthy subjects. During both fasting and duodenal nutrient stimulation, the median proportion of FWs followed by a PAW was lower in patients (0% (0-8%)) than in healthy subjects (36% (11-44%); P<0.001). The volume amplitude of FW in patients was smaller than in healthy subjects (87 ± 8mL vs. 44 ± 3mL; P<0.001). **Conclusions:** In critically ill patients, in addition to decreased motor activity in both proximal and distal stomach, the functional association between proximal and distal gastric motor activity is virtually absent. This incoordination is likely to interfere with the transfer of gastric contents to the distal peristaltic pump and contribute further to the delayed emptying in these patients.

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Changes in gastric emptying and smooth muscle contractility in the db/db mouse model of diabetes

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Abnormal gastric function has been described in patients with diabetes. Different animal models have different pathophysiologic

abnormalities in gastric function. Gastric emptying (GET), a functional test, is usually measured using a gavage technique which may introduce artifact related to stress or anesthesia.
Aims: to determine GET in db/db diabetic mice and their lean littermates using a non-invasive radionuclide technique, and to correlate these findings with glucose levels and with gastric circular muscle response to several contractile and inhibitory neurotransmitters. **Methods:** Db/db diabetic, 21 to 31 weeks of diabetes, and lean littermates were studied. GET was determined by imaging the emptying of Tc-99m sulfur colloid labeled scrambled eggs over 3 hours. Animals were trained to eat eggs and to lie in restraints prior to the study. Animals were sacrificed at least 3 days later and stomachs removed. Muscle strips were mounted in the circular muscle orientation in individual tissue chambers and perfused with Krebs solution, gassed with 95% O2 and 5% CO2. Strips were stretched to Lo. Dose responses of antrum, body and fundus were obtained to carbachol alone, forskolin in the presence of carbachol, KCl and NaF (from 3 to 8 animals). Muscle tension was normalized to cross-sectional area. **Results:** Data is presented as mean ± SEM. The T1/2 for GET was more rapid in diabetic (n=13) than lean mice (n=14) (59.3 ± 5.7 vs. 77.2 ± 5.8 min, p<0.05). Blood glucose levels were 476.9 ± 29 vs. 105.2 ± 4.8 mg/dl respectively. There was no correlation between glucose level and GET. Within both diabetic and lean groups, the contractile responses of the antrum to most doses of carbachol were significantly greater than the responses of the body (p<0.05). In lean animals, but not in diabetics, the antral response was greater than that of the fundus. No significant differences in the responses to carbachol were seen between diabetic and lean groups, with wide variability in responses in diabetic animals. NaF responses paralleled the carbachol responses. In the lean mice the contraction to KCl (75,100,125mM) was greater in the antrum than body or fundus, while in diabetics a difference was found between antrum and fundus only. A shift of the dose response curve to forskolin to the right was seen in the diabetic gastric body, but not the fundus or antrum. **Conclusions:** 1. In the db/db diabetic mice, the stomach emptied solid material more rapidly than in lean littermates and gastric emptying rates were not correlated with glucose levels. 2. The differences in neurotransmitter responses suggest a complex effect of long-term diabetes on signal transduction pathways in the stomach in this model of diabetes.

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Effect of enteral versus parenteral feeding after pylorus-preserving pancreaticoduodenectomy on delayed gastric emptying: Results of a randomized controlled trial

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Background: Delayed gastric emptying (DGE) is a leading cause of morbidity after pylorus-preserving pancreaticoduodenectomy (PPPD), occurring in up to 40% of patients and resulting in significant prolongation of the hospital stay that adds to hospital costs. Postoperative artificial nutritional support has been shown to benefit patients undergoing major gastrointestinal surgery. The purpose of the present study was to compare enteral versus parenteral feeding for reducing the incidence of postoperative DGE. **Methods:** From September 2001 to August 2005, a total of 78 patients with periampullary lesions undergoing PPPD were randomly allocated to receive either enteral nutrition or parenteral nutrition. Exclusion criteria were age <18 years, prior gastric surgery, prior chemotherapy or radiotherapy. Nutritional support, supplying daily 30 kcal/kg and 1.5 g/kg of protein, based on ideal body weight, was provided within 24 hours of surgery. Baseline characteristics were well matched in the two groups. Appropriate written informed consent was obtained from each patient. Statistical significance was accepted at a p value of less than 0.05. **Results:** Enterally fed patients had a significantly shorter duration of nasogastric tube drainage than parenterally fed patients (postoperative day 4.4±0.6 vs. 11.4±0.9). Patients receiving enteral nutrition resumed eating earlier (postoperative day 7.9±2.1 vs. 15.2 ±1.8). The number of days with high-volume (0.5 L) nasogastric tube drainage was significantly less in the enterally fed group. The incidence of DGE was 10.2% in the enterally fed group and 41.0% in the parenterally fed group (p<0.05). Hospital stay was shorter in the enterally fed group (16.1±2.3 vs. 24.5±2.1 days, p<0.05). Cholecystokinin levels were lower in enterally fed group patients, before and after feeding, compared with parenterally fed patients on postoperative day 9 (p < 0.05). **Conclusion:** Enteral feeding after PPPD could be used as prophylactic therapy for DGE since it significantly reduce the incidence of DGE as well as the number of days until patients tolerate an oral diet, thereby reducing hospital stay.

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Effects of Iberogast® on proximal gastric volume, antropyloroduodenal (APD) motility and gastric emptying in healthy men

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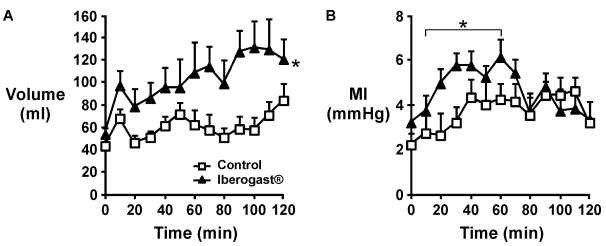
Introduction: The herbal preparation, Iberogast® (''IBE''), improves symptoms in functional dyspepsia. *In vitro* animal studies indicate that IBE has a dual action on the gastrointestinal (GI) tract, eg IBE decreases fundic tone, while increasing antral motility, in muscle strips of guinea pig stomach in a concentration-de-pendent manner (Hohenester et al. Neurogastroenterol Motil 2004;16:765-73). In human gastric muscle pre-parations IBE relaxes the proximal stomach (Schemann et al. Phytomedicine 2006; in press); the magni-tude of this effect is comparable to that observed in the guinea pig stomach. The effects of IBE on the intact human GI tract have, hitherto, not been evaluated. **Hypothesis:** IBE will increase proximal gastric volume (''gastric relaxation''), stimulate phasic pressures in the antrum and duodenum, decrease pyloric pressures, and have little or no effect on gastric emptying, but increase meal retention in the proximal stomach. **Protocol:** We evaluated the effects of oral IBE and control (''C''), each administered as a single dose (1.1 ml - as recommended for IBE), in double-blind, randomized fashion, on (Part A) proximal gastric volume, by barostat, (Part B) APD motility, by high-resolution manometry, and (Part C) gastric emptying and intragastric distribution of a solid/liquid meal, by scintigraphy, in 9 (Part A), 12 (Part B) and 8 (Part C) healthy males. **Results:** IBE increased proximal gastric volume ([ml], C: max volume 103.6±11.6, IBE: max volume 173.8±22.6; P<0.05), and increased the motility index of antral pressure waves in the first 60 min (P<0.05), without affecting pyloric or duodenal pressures. IBE slightly increased the retention of liquid in the total stomach between 10-50 min (P<0.01), although there was no significant difference in the T50% for liquid ([min], C: 19.5±2.5, IBE: 23.1±2.4), and no effect on gastric emptying of solids (retention at 100 min [%]; C: 40.3±3.7, IBE: 42.8±4.7) or intragastric meal distribution. **Conclusions:** These observations establish that IBE has region-dependent effects on gastric motility in humans by relaxing the proximal stomach and stimulating antral motility. These effects may contribute to the reported therapeutic efficacy of IBE in functional dyspepsia.



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Severity of burn-induced delayed gastric emptying in rats

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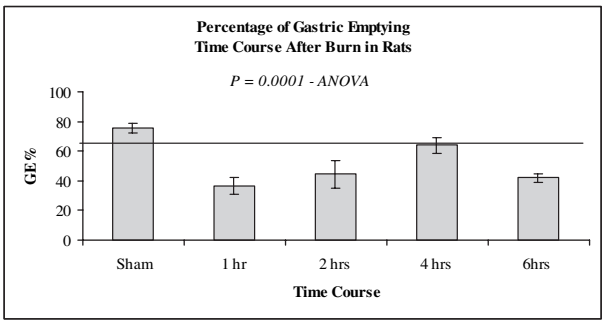
Introduction: Impaired gastric motility has been reported in both animals and humans following severe burn injury. Upper gastrointestinal (GI) ulceration, bleeding, feeding intolerance, abdominal distension, vomiting and ileus constitute major clinical problems. Early enteral feeding (EEF) is an important component of therapy in burned patient and is of great clinical significance. However, EEF is hindered or may have to be delayed due to burn-induced gastric dysmotility. Therefore, it is of great clinical significance to understand the time course in the alteration of gastric motility following severe burn injury, thus to seize the window of opportunity during which gastric motility is normal and EEF can be feasible. **Methods:** Adult male Sprague-Dawley rats received general anesthesia and were prepped to receive a sham or third degree scald burn (40% total body surface area) after 24hrs of food deprivation. Gastric emptying (GE) was studied over a time course after burn; burned rats were randomized into 4 groups: one, two, four and six hours after burn (9 rats each). GE was studied by gavage feeding of a methylcellulose meal mixed with phenol red as a marker. Rats were sacrificed 30 min after the meal, their stomachs were harvested and the amount of phenol red recovered was measured. Calculations of GE were expressed as percentage of meal emptied after 30 minutes. **Results:** GE% was significantly delayed at one, two and six hours after burn versus sham (GE% were: 36.9±5.6, 44.6 ±9.4 and 42±8 vs. 75.6±3.4, p=0.00002, 0.01 and 0.000001 respectively). However, GE% at 4 hrs after burn was not significantly different from the sham group. **Conclusion:** After burn, GE is severely impaired. However, it gradually recovers overtime, reaching its best at four hours after burn. Four hours after burn may represent the best opportunity in which EEF can be administered safely. Further studies are needed to investigate the mechanisms responsible for the GE recovery after burn.



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Percutaneous Endoscopically-controlled Gastrostomy (PEG) tube as a mean to place electrodes for gastric pacing without surgery: safety and feasibility

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Introduction: Gastric electrical stimulation (GES) or pacing involves the surgical placement of gastric electrodes under anesthesia. Placing gastric electrodes through upper GI endoscopy is an attractive method that can diminish the risk of surgery in gastroparesis and obese patients. The aim of this study was to investigate the safety and feasibility of placing stimulation electrodes endoscopically for GES as well as to test the efficacy and efficiency of these electrodes for treatment of gastroparesis and obesity. **Materials and Methods:** A pair of gastric pacing wires was attached to a PEG and inserted endoscopically at 10-15cm above the pylorus in four female hound dogs. Following endoscopy, dogs underwent laparotomy in which four pairs of cardiac pacing wires were placed on the serosal surface along the greater curvature at 2, 6, 10 and 14 cm above the pylorus. The location of either the first or the second proximal pair was adjusted to be circumferentially aligned and 3-5cm to the PEG electrodes. After recovery, GES was introduced using either the PEG or the serosal electrodes. For entrainment of gastric slow waves, long pulse GES was applied at a frequency 10% higher than the intrinsic frequency of gastric slow waves. To study the potential of PEG electrodes in the treatment of gastroparetic symptoms, vomiting was induced by vasopressin infusion (0.5U/Kg). Short pulse was applied through PEG/serosal electrodes to reduce vasopressin-induced emesis. Assessment of signs was observed during baseline, vasopressin infusion and recovery periods. To study the potential of PEG electrodes in the treatment of obesity, long pulse GES was introduced at a tachygastrial frequency (20cpm) to induce dysrhythmias and possibly symptoms. **Results:** 1) Endoscopically-placed electrodes were capable of recording gastric slow waves, comparable with the serosal electrodes. 2) Entrainment of gastric slow wave was achieved by long pulse GES on PEG electrodes at a lower energy than serosal electrodes (300-600ms, 3-6mA vs. 300-600ms, 6-10mA). No signs were observed while GES was performed using the PEG electrodes. 3) Improvement of vasopressin-induced signs was achieved through short pulse GES on PEG electrodes of 300μsec, 2mA and a frequency of 20cpm, during both infusion and recovery periods. 4) Induction of dysrhythmias and vomiting was achieved through long pulse GES on PEG electrodes at a higher energy than serosal electrodes (300-500ms, 7-10m vs. 300ms, 6-7mA). **Conclusion:** Endoscopically-placed pacing electrodes are both feasible and safe. PEG electrodes are effective and have a potential for use in treatment of both gastroparesis and obesity.

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Influence of the caloric content of a liquid nutrient on partial gastric volumes and upper abdominal sensations in patients with functional dyspepsia

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Background and aims: Functional dyspeptic (FD) patients complain about a variety of symptoms, which are mostly related to food intake. In order to reduce postprandial symptoms, many patients reduce the caloric content of a single meal, however the rational of this is not fully understood. The aim of the study was to evaluate the influence of the caloric content of a liquid nutrient on postprandial abdominal sensations, and to relate these sensations to the change in total and partial gastric volumes. **Methods:** Fourteen FD patients (10 women, 4 men; mean age 46 years; range 20-62) with severe postprandial upper abdominal symptoms were included. A three-dimensional ultrasonographic (3D-US) study of the stomach was performed twice, on two separate study days, after either a high-caloric (500 ml, 300kcal) or a low-caloric (500ml, 20kcal) liquid nutrient. Total, proximal, and distal gastric volumes were assessed while fasting and at 5, 15, 30, 45, and 60 minutes postprandially. At all consecutive time points, upper abdominal sensations (satiation, fullness, nausea and upper abdominal pain) were scored using a visual analogue scale (VAS). **Results:** Total gastric volume was larger after the high-caloric meal (Anova: $P < 0.001$). In addition, proximal and distal gastric volumes were significantly larger after the high-caloric meal compared to the low caloric meal (Anova: $P = 0.001$ and $P = 0.039$ respectively). After the high-caloric meal, satiation scores were higher compared to the low-caloric nutrient ($P = 0.030$). An increase in upper abdominal sensations occurred both after ingestion of the high-caloric and the low-caloric nutrient (all $P < 0.05$). However, postprandially, no differences in fullness ($P = 0.076$), nausea ($P = 0.866$), and upper abdominal pain ($P = 0.688$) were observed between the high-caloric and low-caloric nutrient. No significant correlation was found between partial gastric volumes and upper abdominal sensations, either after the high-caloric or the low-caloric nutrient. **Conclusions:** The caloric composition of a meal affects gastric motor function. This effect on gastric motility is independent from the effect on visceral perception in functional

dyspepsia. Therefore, the caloric content of a meal cannot be regarded as a specific trigger for dyspeptic symptoms.

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In post-surgical gastroparesis - gastrointestinal electrical stimulation improves symptoms independently while gastric emptying response is dependent on baseline emptying

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Introduction: Temporary GES has previously been shown to improve both gastric emptying and symptoms in post-surgical (PS) gastroparesis (GP) but long-term effects on GI symptoms and gastric emptying are unknown. Many post-surgical gastroparesis patients have non-delayed emptying, and long-term effect on baseline normal or rapid emptying is also unknown. Patients: 36 pts (6 M, 30 F, age 46±2 yrs) with post-surgical (Bilroth I, n=11; Bilroth II, n=4; other, n=21) disordered gastric emptying were evaluated. **Methods:** GI symptoms (nausea=N, vomiting=V, Total= TSS) and solid gastric emptying (GET at 1, 2, 4 hrs) were compared at baseline (Base) with temporary (Temp) and permanent (Perm) GES (previously described:NGM, 2004; 16: 635.) Long-term follow-up results (6 mo to 10 yrs) are reported as mean ± SEM and are compared by t-tests. **Results:** Of the 36 patients, 29 (20 delayed and 9 non-delayed) had valid baseline emptying, the others not tolerating food at baseline. GI symptoms improved from baseline with both Temp and Perm GES (nearly all $p < 0.05$). GET for both Temp and Perm GES accelerated for delayed patients and generally slowed for non-delayed ($p<0.05$ for 1 hour values). See tables below. **Conclusions:** In a large group of post-surgical GP patients, both Temp and Perm GES improved GI symptoms independently of gastric emptying and for prolonged time. The effect of GES on GET is independent on symptoms improvement but is dependent on baseline gastric emptying.

Delayed	1 hour	2 hours	4 hours
Baseline	80 ± 5	72 ± 6	57 ± 6
Temp GES	84 ± 6	69 ± 7	50 ± 9
Perm GES	80 ± 5	62 ± 5	45 ± 6
Rapid	**1 hour**	**2 hours**	**4 hours**
Baseline	14 ± 5.3	3.9 ± 0.3	3.2 ± 1.1
Temp GES	35 ± 14	16 ± 7.0	3.5 ± 1.7
Perm GES	38 ± 8.6	12 ± 5.4	4.0 ± 1.9

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The relationship of antropyloroduodenal motility in diabetic gastroparesis and response to intrapyloric botulinum toxin injection

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Background: Intrapyloric injection of botulinum toxin (BT) has variable efficacy in the treatment of gastroparesis. Recently, we showed that pylorospasm (basal pyloric pressure >10mmHg) is present in approximately 60% of patients with diabetic gastroparesis, which may explain the variable response rate. However, even when selecting patients with elevated pyloric pressure (PP), BT injection has not led to a reliable clinical response. **Aims and Hypothesis:** The aim of our study is to compare antroduodenal motility(ADM) patterns in patients with diabetic gastroparesis and pylorospasm who had a symptomatic response compared to those without a clinical response to BT injection. Our hypothesis is patients unresponsive to BT have other motility abnormalities as the major cause of their symptoms. **Methods and Patients:** 15 patients with diabetic gastroparesis (F=9, M=6; mean age 41.2 ± 3.1 years) with symptoms refractory to standard medical therapy were evaluated with nuclear scintigraphy and antropyloroduodenal manometry. All patients had delayed gastric emptying (mean 4hr retention 45.7 ± 6.2%) and pylorospasm (mean basal PP 22.5 ± 3.0 mmHg). Stationary pyloric manometry was performed using a water perfused Dent sleeve prior to injection with BT 100U. ADM was performed during 3 hours of fasting and following stimulation with erythromycin and octreotide. Statistical analyses were perform using the t-test and Fisher's exact test. **Results:** 46.7%(7/15) patients had partial to complete (> 50%) symptomatic improvement following BT injection. There were no significant differences in the 4hr gastric retention in either group before or after BT injection. Overall, there was also no change in the gastric retention after BT injection. 7/15 patients had an absence of the phase 3 of the MMC in the stomach and duodenum during fasting, 6/15 antral hypomotility, 1/15 normal antral contractions and 1/15 tachygastria on their ADM at baseline. There were no differences in fasting ADM pattern in patients who responded to BT compared to those who did not. However, all patients (7/7) with symptomatic improvement had antral contractions stimulated with erythromycin. In comparison, only 25%(2/8) of patients who did not respond to BT injection had antral contractions stimulated with erythromycin ($p=0.002$). **Conclusions:** 1)Absence of the antral component of the phase 3 of the MMC was found in the majority of patients with diabetic gastroparesis. 2)The presence of antral hypomotility does not predict a poor response to BT injection. 3)However, the inability to stimulate antral contractions with erythromycin does predict a poor response to BT injection.

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Tegaserod improves intragastric food distribution and solid gastric emptying of FD patients

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Background: Previous studies show tegaserod (T) has a prokinetic effect on healthy subjects and patients with gastroparesis. It remains unknown whether T can influence intragastric food distribution (IGFD) and proximal gastric emptying. **Aim:** To investigate whether T (6mg bid 14d) can improve IGFD and solid gastric emptying (including proximal and global stomach) of FD patients by a randomized, double-blinded, placebo-controlled clinical trial. **Method:** 38 FD patients (26F, mean 43yrs, 19 patients in treatment and control group, respectively) participated in the study and underwent the radionuclide gastric emptying test twice before and after treatment. The test meal includes 80g noodle, 50g sausage and 50g scrambled egg containing 0.5mCi of ^{99m}Tc-SC (584Kcal). The γ camera (Mellennium Hawkeye, USA) was used with 140KeV power peak, 50% window width, 1.5 zoom and low-energy parallel hole collimates for all purpose. 7 images were obtained at 0, 15, 30, 45, 60, 90 and 120min after test meal intake with each one being viewed for 1min. Regions of interest (ROI) including global and proximal stomach were outlined in computer automatically and manually. The division of proximal and distal stomach was according to mid-gastric transverse band. IGFD was expressed with the ratio of radio counts in proximal and distal stomach (P/D) including seven collection points and area of under curve (AUC) of the time-IGFD curve. Parameters of gastric emptying include Lag Phase (LP), Half Emptying Time ($T_{1/2}$), Emptying Velocity (EV) and Residual Percentage in 120min (RP). **Results:** In baseline, no significant difference was found in any parameter between the two groups. (2) After treatment, P/D of T group was higher than control group at 0min (5.6±2.9 vs. 4.4±2.3), 15min (4.5±2.4 vs. 3.5±2.2) and AUS (435.8±225.0 vs. 375.0±180.0) (P 0.05), however, P/D at 30, 45, 60, 90 and 120min were similar between the two groups. (3) Compared with control group, significant difference existed in $T_{1/2}$ of global stomach (116.0±32.4 vs. 140.5±38.4min) and EV of global and proximal stomach (0.60±0.17 vs. 0.48±0.16 & 0.58±0.16 vs. 0.47±0.11%/min) between the two groups (P 0.05), but not in $T_{1/2}$ of proximal stomach, LP and RP. **Conclusion:** T improves intragastric food distribution of FD patients by making more food distribute to proximal stomach. T has a prokinetic effect on solid gastric emptying by shortening $T_{1/2}$ of global stomach and EV of global and proximal stomach. The results above suggest T may be useful to FD patients with abnormal intragastric food distribution and impaired proximal and global gastric emptying.

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Comparative effects of intravenous fructose and glucose on gastric emptying and antropyloroduodenal motility in healthy subjects

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Acute elevations in the blood glucose concentration, even within the normal postprandial range, slow gastric emptying (GE) and affect gastric motility, in both healthy subjects and patients with diabetes mellitus. Fructose is used widely in the diabetic diet and is known to empty from the stomach slightly faster than glucose. The aims of this study were to evaluate the effects of intravenous (*iv*) fructose compared with *iv* glucose on GE and antropyloroduodenal (APD) motility. Six healthy males (mean age 26.7±3.8yr) underwent concurrent measurements of GE of a solid meal (100g ground beef labeled with 20MBq ^{99m}Tc-sulfur colloid) and APD motility on three separate, randomized days during *iv* infusion (20min) of either fructose (0.5g/kg), glucose (0.5g/kg) or isotonic saline. GE (scintigraphy), APD motility (manometry) and blood glucose (glucometer) were measured for 120min. Intravenous glucose and fructose both slowed GE substantially (Fig a) (P<0.005 for both), without any significant difference between them (there was a trend (P=0.07) for faster GE with fructose compared with glucose at 45min). There were more (P=0.02) isolated pyloric pressure waves (IPPWs) at 15min with glucose compared to fructose (Fig b). There was a rise in blood glucose (P<0.001) after glucose, but not after fructose or saline (Fig c). Only 3 of the 6 subjects experienced mild nausea with fructose, which resolved after completion of the infusion.

In conclusion, iv fructose slows GE in healthy subjects; the magnitude of this effect appears comparable to glucose.

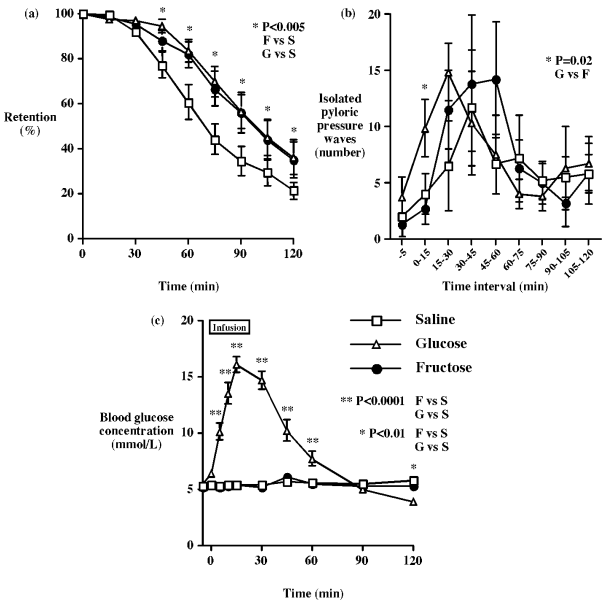

Figure 1 Effects of iv fructose, glucose and saline on (a) GE, (b) IPPWs and (c) blood glucose, following ingestion of 100g ground beef. Data are mean ± SEM.

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Effect of a Nutrient Drink Load Test on Gastric Myoelectrical Activity and Upper Gastrointestinal Symptoms in Healthy Adults

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Background: Nutrient drink load tests are frequently used to assess maximum tolerated volumes and satiety in healthy volunteers and patient populations. **Aim:** The aim of this study was to test the effects of a nutrient drink load on gastric myoelectrical activity, as measured by electrogastrography (EGG), and upper gastrointestinal symptoms in healthy adults. **Methods:** Forty-one healthy adults (21 male, 20 female; age range = 20-62 years, mean age = 32.6 years) were enrolled and completed the nutrient drink load test. Each participant reported to the laboratory after an overnight fast. The nutrient beverage consumed was Ensure® (22% fat, 14% protein, 64% carbohydrate). Each participant drank 4 oz of Ensure® every 4 min until maximum satiety was reached. Participants rated on 10 mm visual analog scales (VAS) their symptoms of nausea, fullness, and bloating; ratings were made at baseline, at the point of maximum satiety, and 30 min after satiety had been reached. EGGs were recorded using standard methods and analyzed for a 15-min baseline and for 30 min after satiety had been reached. **Results:** The average volume of Ensure® ingested to achieve satiety was 1078.8 ml. Of the 41 participants, 37 reported nausea after satiety, and three vomited. VAS ratings of nausea were significantly higher during the 30 min after satiety had been reached (mean = 3.0) compared with baseline (0.1), $t(40)=7.82$, $p<.001$. Likewise, ratings of fullness were significantly higher after satiety (7.4) than during baseline (0.4), $t(40)=21.32$, $p<.001$. Ratings of bloating were also significantly higher after satiety (4.4) than during baseline (0.1), $t(40)=10.51$, $p<.001$. Compared with baseline, tachygastria increased significantly from baseline to the first 30 minutes after satiety (27% to 33%), $t(38)=2.69$, $p=.01$. Bradygastria decreased significantly in the first 30 min after satiety (41% to 35%), $t(38)=-1.97$, $p=.05$. No change was observed in normal gastric activity over the same period of time (23% to 23%). **Conclusions:** The Ensure® nutrient drink load test induces fullness but also nausea, bloating, and gastric dysrhythmias in healthy adults. The nutrient drink load test represents a model for induction of postprandial dyspepsia symptoms and gastric dysrhythmias. This study was sponsored in part by Novartis Pharmaceuticals.

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Usefulness of long time assessment in scintigraphic gastric emptying

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Background & Aim: Gastric emptying (GE) test is usually performed with a short observation time, i.e. 3 or 4 hours and GE kinetic is reduced to a set of few parameters such as Tlag, Half emptying time T½, Fraction of solid phase remaining at 2, 3 or 4 hours, by means of direct or computed measurement using mathematical model. This protocol, which assumes that GE unfolds in a regular manner, is sufficient for rapid or normal gastric emptying . In case of gastroparesis T½ is longer than 4 hours. For many functional patients the onset and the end of symptomatology in the day suggest that GE is not impaired in its duration but in its chronology or sequencing. We assessed GE for at least 6 hours to try to understand in individual cases of functional disorders or gastroparesis the relationship between symptomatology and digestive tract motility. **Patients and Methods:** 15 patients (idiopathic neuropathy, diabetes mellitus, idiopathic dyspepsia) are seen between 1:00 PM and 10:00 PM for per and postprandial phase of a typical 800 kcal French lunch regularly interrupted by the intake of 8 pieces of hard-boiled egg white radiolabelled with Tc99m sulfur colloid. Scintigraphic imaging is performed patient upright in dynamic mode for 1 min at 1 frame/1s during tracer intake then at intervals of 30 min in static mode anterior/posterior view for GE until 07:00 PM when they have dinner. Then we are looking for action of the new intake upon lunch radiolabelled GE and eventuality of meal cross-over, i.e. mixing midday meal residues and actual meal. We quantify intragastric distribution of meal per and postprandially. Fraction of meal remaining in the stomach is expressed as percent of actual total counts. For combined evaluation we also study antral motility by imaging during 4 min at 1 frame/4s at intervals of one hour. **Results:** In healthy subjects GE of this 800kcal test meal is completed within 4-6 hours comprising an initial Lag Phase of about 2 hours, meal duration included. There are no residues by dinner time. Among diabetic patients there is one case of rapid, near normal GE,

one case of typical gastroparesis with 60% remaining, so that GE at dinner postprandial time concerns midday meal ; there are some cases of multiphasic GE with a lag-phase between an early and a late emptying phase, while most cases showed delayed GE with 1 to 15% residues recycling with the next meal in the whole stomach or just in the antral part in cases of impaired trituration.**Conclusion:** This protocol allows us to grade the severity of gastroparesis, to find events of maceration with residues mixing from one meal to another, to assess digestive neuropathy and define customized chronology of intestinal nutrients delivery with the possibility of delayed partial dumping episodes even among diabetic patients.

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Mucosal amplitude ratio of temporary EGG (MART) predicts outcome of response to gastric electrical stimulation

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Introduction: Gastric Electrical Stimulation (GES) is an accepted therapy for gastroparesis (GP) with chronic nausea and vomiting. Temporary GES is a new approach applying GES endoscopically via the gastric mucosa. We previously reported that the ratio of frequency to amplitude of the serosal EGG at the time of placement might correlate with outcome of permanent GES. We investigated the hypothesis that the ratio of frequency to amplitude of the mucosal EGG (mEGG) at the time of temporary GES placement might predict outcome to therapy. **Patients:** 150 Patients, (119 F, 31 M, mean age 44 yrs) with the symptoms of gastroparesis and Diagnosis: 83 Idiopathic, 41 Diabetic, and 26 Post-surgical underwent temporary GES (TempGES) as previously described (GIE, 2005). **Methods:** Patients were assessed with baseline and after 3 days of TempGES in terms of symptoms (Sx) of Vomiting (V), Nausea (N), and GI Total Symptom Score (TSS) The average Frequency (F), Amplitude ratio of the temporary EGG (MART) was calculated and compared with the outcome of Temp GES in (A) and Mucosal (frequency to) Amplitude terms of % change of Sx: N, V, and TSS as well as GET by Total Gastric emptying (sum of 1, 2, 4 hour GET). Results were compared by t-test and correlation and reported as mean ± SE. **Results:** After 3 days of TempGES, Vomiting score changed from 2.3 ± 0.1 to 0.5 ±0.1, nausea from 3.3± 0.1 to 1.2 ±0.1, and TSS changed from 14.3±0.3 to 5.3±0.4 (all $p<0.05$ by paired t-tests). The correlation of mEGG Frequency alone ($p=0.11$) or Amplitude alone ($p=0.33$) to V were not significant alone. The correlation of the mucosal frequency to amplitude (MART) significantly correlated with percent change in Vomiting ($p<0.001$). Gastric emptying improved in most patients (mean 54%) and MART also correlated with % change in total GET: $r= 0.7$, $p < 0.0001$. **Conclusions:** We conclude that the use of mucosal recording of the EGG at the time endoscopic mucosal electrode placement (MART) correlates with both symptom and gastric emptying outcomes of Temporary GES. The use of MART may assist in determining which patients with GP may benefit from permanent GES.

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Patterns of gastric relaxation in response to intake of solid food, nutrient drink and non-nutrient drink

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Gastric relaxation upon food ingestion is a well-known physiological phenomenon. However, it is unknown whether this relaxation is associated with the types of ingested food.

Aims: of this study were to 1) to study the patterns of gastric relaxation in response to intake of solid food, nutrient and non-nutrient drink, measured by barostat. 2) To study the effects of the ingested volume/calories on the pattern of gastric relaxation. 3) To differentiate various patterns of gastric relaxation and to assess the accuracy of the detection of food intake using barostat. **Methods:** Six female dogs equipped with a gastric cannula were involved in this study. The study was composed of a number of randomized sessions, each consisting of two periods. The animal was fasted overnight before the study. A barostat balloon was positioned inside the stomach to record gastric volumes. After a 20 minutes baseline recording, the dog was fed with different food/drink with different volumes (water at volumes 50ml or 100ml, coke at volumes 50ml or 100ml, can food at 50g or 100g) and the recording after each intake was continued for 20 minutes. **Results:** Intake of solid food, coke but not water caused gastric relaxation. Gastric volume was 55.7±9.7 ml at baseline, 137.7±25.2 ml with 50g solid ($P<0.01$ VS. baseline) and 144.3±28.0 ml with 100g solid ($P< 0.01$ VS. baseline); Gastric volume was 58.3±13.4 ml at baseline, 147.9±36.9 ml with 50ml coke ($P<0.01$ VS. baseline) and 156.2±26.5 ml with 100ml coke ($P<0.01$ VS. baseline); Gastric volume was 81.2±5.9ml at baseline, 113.1±10.0ml with 50ml water ($P=0.21$ VS. baseline) and 94.1±6.5 ml with 100ml water ($P=0.08$ VS. baseline); 2) There was a significant difference in the change of gastric volume between water and nutrient drink or solid food. The change of gastric volume was 23.2±6.2ml during water intake, 90.1±12.6ml during coke intake ($P<0.01$ VS. water intake) and 86.0±15.1 ml during solid food intake ($P<0.01$ VS. water intake). 3) The volume/calories didn't result in significant difference on the pattern of gastric relaxation. **Conclusions:** Water intake does not induce a sustained and substantial increase in gastric volume, suggesting the possibility of differentiating water intake from others. Nutrient drink and solid food intake have similar effects on gastric volume. These data suggest that nutrient intake (whether solid or liquid) can be detected from the measurement gastric distention.

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The influence of maternal functional bowel disorders on childhood abdominal pain and somatic and psychological co-morbidity

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Abdominal pain (AP) of unknown origin is a common medical complaint that is associated with a variety of somatic (headache, limb pain, back ache) and psychological (anxiety and depression) co-morbid symptoms both in children and adults. Previous studies have demonstrated that the children of parents with IBS have more physician visits for GI as well as non-GI clinic visits over a period of 3 years. **Aim:** To examine the patterns of presentation for AP, somatic and psychological co-morbidity from childhood to adulthood in the offspring of mothers with FBD. **Methods:** A birth cohort of all children born between 1976 and 1982 to mothers who were residents of Rochester, MN, and who remained in the community until age 5 years, was considered for this study. Medical visits for a FBD in mothers and AP of undetermined etiology in their children and somatic and psychological co-morbid conditions (above) in their children were identified by HICDA codes. A database of the medical providers for which 95% of the county residents receive health care was used for this study. Subjects were followed based on their diagnoses accumulated while the child was <21 years. A group of age and gender matched controls of children without a diagnosis of AP before ages 21 were used to compare maternal FBD and co-morbid conditions. 80% of the population remained in the area until age 19. **Results:** Of the 5347 birth cohort members without AP prior to age 5, 1358 individuals had an incident medical visit for AP of unknown origin from 5 to <21 years (single visit only 61%, 2 visits 21%, 3+ visits 18%). Of these children, 47% of their mothers had a FBD diagnoses when their child was between birth and <21 years of age. Children with one or more medical visit for AP had a progressively increased risk of having a mother with a FBD diagnosis (OR = 1.39 (95% CI, 1.29-1.49) per 1 unit change in number of visits, all $p<0.001$). Children who presented with AP with a mother who had a diagnosis of FBD were more likely to present with each of the somatic (head ache, back pain, limb pain) and psychological (anxiety and depression) co-morbid conditions compared to a child with AP with a mother without a FBD diagnosis (O.R. range between 1.3-1.7, all $p<0.05$), and compared to a child without AP and without a maternal FBD diagnosis (OR range between 2.7-4.6, all $p<0.001$). **Conclusion:** Maternal FBD and childhood AP medical presentation frequently co-occur. Maternal FBD is also associated with co-morbid medical complaints in the child who presents with AP from childhood to early adulthood.

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Effects of water ingestion on gastric electrical activity and heart-rate variability in healthy children

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The purpose of this study was to investigate the effects of water loading on heart rate variability (HRV) and electrogastrogram (EGG) parameters in healthy children and correlation between HRV and EGG parameters. **Methods:** Twenty-eight healthy children (14M, 14F, mean age:12.5 years, range: 8-17) underwent simultaneous recordings of EGG and electrocardiogram (ECG) for 30 minutes in the fasting state and 60 minutes after ingestion of water to maximum satiety within 3 minutes (water loading). Quantitative EGG parameters were calculated by spectral analysis method. The HRV signal was derived from the ECG recording and the power in the high-frequency band (0.15 to 0.50 Hz) before (pre-HF) and after water ingestion (post-HF) and their ratio (rHF =post-HF/pre-HF) were calculated by power spectral analysis of HRV signal. Data are presented as mean ± SE. **Results:** The mean water ingestion to maximum satiety was 513cc. Water loading resulted in a postprandial increase in EGG dominant power ($P<0.05$) and a decrease in EGG dominant frequency (2.95±0.06 cpm vs. 2.69±0.08 cpm, $P<0.05$ for the first 30-min postprandial period) but had no effect on percentage of normal slow waves. Spectral analysis of HRV showed no significant changes in any HRV parameters after water loading. The first 30-min postprandial EGG power increase was significantly correlated with post-HF ($r=0.449$, $P=0.01$) and rHF ($r = 0.608$, $P= 0.002$) in the first-30 postprandial period but not in the second 30-min postprandial period. **Conclusions:** Water loading resulted in a significant increase in postprandial EGG power for up to one hour and a significant decrease in EGG dominant frequency but no effect on slow wave regularity. The postprandial EGG power increase was significantly correlated with the ratio of post-HF to pre-HF and post-HF, suggesting that water loading enhances the gastric electrical activity mainly through vagal activity in the immediate postprandial period.

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Gastric electrical stimulation for adolescents with intractable nausea and gastroparesis

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Purpose: Gastric electrical stimulation (GES) has been performed in adults as a treatment for refractory nausea and vomiting, in patients who have failed medical treatment. It has not been systematically applied to individuals less than 18 years old with this problem. **Methods:** Six patients, One male, and Five females with chronic nausea and vomiting with a mean age of 15 years (range 13-18), were evaluated with gastric emptying studies and cutaneous electrogastrogram (EGG) for temporary GES. All patients had idiopathic gastroparesis. Five patients subsequently underwent placement of a permanent GES device - four were done laparoscopically and one was open. Each patient had an intra operative EGG and in three patients, sero-muscular biopsies from the stomach or jejunum were obtained as well. Symptoms were recorded at baseline, after temporary pacing and then after permanent pacing using a Likert scale (0-4 for each symptom with a total of 5 symptoms). Statistical analysis was performed using a paired student's t test and a value of <0.05 was considered significant. **Results:** At baseline, all patients were symptomatic and most had delayed solid gastric emptying and abnormal EGG. As a group, there was a significant improvement in nausea (3.4 ±0.4 to 1.7±0.3, $p=0.005$), and combined symptoms score (11.3±2.0 to 5.0±1.5, $p=0.02$). Gastric emptying and EGG values also improved. Biopsy was abnormal in 2 of 3 patients, showing diminished and abnormal Cajal cells in the two patients who had less improvement long term. Follow up ranged from 1- 20 months, with an average of 9 months. **Conclusions:** GES can be successfully applied to adolescents with intractable nausea and gastroparesis symptoms. The role of the cells of Cajal in enteric motility needs investigation. Long term efficacy of this therapy in children needs to be established.

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Proximal GER events do not predict aspiration in children

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Hypothesis: GER events to the proximal esophagus do not predict chronic aspiration, as indicated by presence of lipid-laden macrophages on BAL or chronic changes in the lungs on HRCT

1. Correlate proximal reflux events by impedance to lipid laden macrophage index (LLMI).

2. Determine association between elevated LLMI, proximal GER, and chronic lung disease on high resolution chest CT (HRCT).

Methods: 1. Subjects: All children who have undergone impedance and BAL and a formal dysphagia study, due to suspicion of chronic aspiration. 2. Excluded if have had positive FEES or VSS, if they cannot aspirate (grade 4 tracheal stenosis), if have CF, immunodeficiency, or BAL culture positive for *Mycobacterium* or fungus. 3. Review pathology slides and calculate LLMI based on method described by Corwin and Irwin. 4. Review impedance results and calculate index of proximal reflux events per hour. 5. Identify cases of chronic lung injury by HRCT based on staff radiologists' impression. 6. Statistics: a). Calculate coefficient of correlation between LLMI and proximal GER events (PEi) (Correlation). b). Compare mean index of proximal events for those with LLMI >90 and < 90.(Student's t test). c). Determine association of LLMI >90, PEi, gender, and age on chronic HRCT changes (correlation, logistic regression, Fischer's exact test). **Results:** 80 subjects identified (mean age: 36 mos, range: 2mos-12yrs; males: 44) LLMI: Mean 20 (3.2), Range 0-145, IQR 2-24, 95%=64; Proximal GER index (PEi): Mean 0.56 (0.09), Range 0-3.44, IQR 0.09-0.70, 95% 1.61; HRCT: 61 with CT performed, 12 read as consistent with chronic aspiration (20%). Spearman Rank Correlation: LLMI with PEi : 0.061, P = 0.59; Mean PEi for LLMI >90 : 0.68; Mean PEi for LLMI <90 : 0.50, P=0.74 PEi with HRCT: 0.139, P= 0.28 LLMI with HRCT: 0.035, P=0.78

Logistic Regression: PEi:OR=1.2P=0.82; LLMI: OR=1, ns; Neutrophil%: OR=1, ns; Age (months): OR=0.9, nsCase control: Fischer's exact test: P=0.82

Conclusion: Proximal GER events do not predict aspiration in children who have intact airway protective reflexes but still have chronic respiratory symptoms.

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Evaluating efficacy of acid suppression therapy in infants

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Background: There is currently no data on the efficacy of acid suppression therapy in infants. **Hypothesis:** Acid suppression therapy (at currently prescribed doses) in infants with suspected GERD, does not significantly alter the characteristics of GER events. **Methods:** We reviewed 30 consecutive pH-impedance data on infants 1 to 12 months (mean 3.8 months, 20 males) suspected of having GERD over a 12 month period. The subjects were admitted overnight to record a minimum of 20 hours of continuous study using simultaneous pH-impedance testing. During the study, 15 subjects were fed via a nasogastric tube (continuous feeds) and 15 were fed orally (PO). 13 were on a proton pump inhibitor (PPI, 0.5 to 1.5 mg/kg/day, 9 on H2-blocker (H2B, 2-6 mg/kg/day) and 8 on no acid suppression medications (No Meds). **Results:** In the orally fed group there was a trend towards more GER episodes recorded in subjects on acid suppression than those that were not ($p=0.09$). Despite similar number of total reflux episodes, subjects on a PPI showed a trend towards less acid reflux episodes than those on H2B ($p=0.09$). Subjects on a PPI had fewer proximal GER episodes than those on H2B or no medications but this was not statistically significant ($p=0.15$). In the NG (continuous) fed group there were significantly fewer GER episodes noted than in the orally fed group ($p=0.03$). Despite continuous NG feeds and acid suppression, the proportion of acid reflux episodes remained essentially unchanged. There were significantly fewer GER episodes in the group treated with either acid suppression regimen than those that did not ($p=0.04$). Despite fewer GER episodes, the H2B treated group had a higher proportion of

acid reflux episodes than those treated with a PPI. Subjects on PPI showed a trend towards fewer proximal GER episodes than those on H2B or no medications (p=0.26).**Conclusion:** At the currently prescribed dosing regimen, our pilot data suggests that H2B have poor efficacy while PPI have borderline efficacy in the treatment of GERD in infants.

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Long term bowel function in children with Down syndrome and Hirschsprung's disease

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Background: The association of Hirschsprung's disease (HD) with Down syndrome (DS) is well documented. There is limited data on the long term bowel function in patients with DS and HD, subsequent to surgical correction. Although some authors have noted higher rates of bowel dysfunction in patients with DS and HD as compared to HD alone, others have not. These series have however primarily included all patients with HD (some with DS) and report that 26% of patients with HD require medications to optimize bowel function on long term follow up. **Aim:** To evaluate long term bowel function and its management in children with DS and HD, and compare our data with 4 similar published studies. **Methods:** IRB approval was obtained for a retrospective chart review of 17 patients who met the diagnosis of both HD and DS between 1979 and 2005. All of the children included in the study had their surgeries at Cincinnati Children's Hospital Medical Center. **Results:** Number=17, Males=12, Rectosigmoid involvement=80%, Duhamel=8, Soave=9, Primary pull through=3, Other congenital anomalies=8 (GI=3, cardiac=7). **Summary:** In our series, on long term follow-up, about half the children with Down syndrome who had definitive surgery for Hirschsprung's disease were still on laxatives. **Conclusion:** Long term bowel dysfunction in children with DS and HD after surgical correction appears to be significantly more common than in those with HD alone. Appropriate counseling and close follow up can potentially greatly facilitate optimal care to this subset of medically complex children.

Mean duration of follow up: 75 months

Laxative use at last visit	8 (47%)
Laxative and enema use at last visit	4 (24%)
Never used laxatives or enema	2 (12%)
Barium enema for constipation evaluation	4 (24%)
Emergency room visit for constipation	8 (47%)
Admission for bowel clean out	2 (12%)

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Effects of various acute stresses on gastric myoelectrical activity and gastric tone in dogs

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Background: Acute stress impairs gastrointestinal motility, such as delaying gastric emptying and altering intestinal transit. However little is known on the effects of various acute stresses on gastric tone and gastric myoelectrical activity. **Aims:** To assess the effects of various acute stresses on gastric tone and gastric myoelectrical activity in a canine model. **Methods:** The study was performed in six dogs implanted with a gastric cannula and one pair of gastric serosal electrodes along the greater curvature. Three forms of acute stresses were applied and described as follows: visual stress: using a specific black eye's cover blinded dog's eye for 1 min, followed by 1 min interval without eye's cover, then repeated the procedure; cold stress: putting dog's leg into ice-water (4^0C) for 1 min, taking out leg from ice-water and putting into warm –water (37^0C) for 1 min interval, then repeated the procedure; audio stress: a tape containing loud household noise (70-80db) was played to each dog via a headphone. Each session was composed of 30-min baseline, 30-min during stress and 30-min recovery without stress. Gastric myoelectrical activity (GMA) was recorded from the gastric electrodes using a recorder with a cut-off frequency of 35 Hz. Gastric tone was assessed with an electrical barostat. **Results:** (1) Acute visual stress did not alter gastric tone (gastric volume from 97.8 ± 11.0 ml at baseline to 113.3 ± 17.9 ml with stress, p>0.05), or gastric slow wave frequency, amplitude or regularity. The slow wave frequency was 5.10 ± 0.11 cycles/min (cpm) at baseline and 5.08 ± 0.11 cpm during stress (P>0.05). (2) Acute cold stress (ice water) and audioc stress (noise) significantly increased the gastric volume from 107.2 ± 13.5 ml baseline to 135.6 ± 23.8 ml with cold stress (p=0.041), and from 106.4 ± 5.7 ml at baseline to 159.2 ± 15.1 ml with audio stress (p=0.007). Although the dominant frequency and power of slow waves did not significantly change during stress, normal percentage of slow waves markedly reduced from 98.3 ± 0.8 to 87.5 ± 3.7 in cold stress session and from 90.2 ± 3.3 to 80.6 ± 2.9 in audio stress session (p<0.05). **Conclusions:** The acute cold and audio stresses inhibit gastric tone and reduce the percentage of normal slow waves. However, the acute visual stress does not seem to have such effects.

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Esophageal motor abnormalities in patients with eosinophilic esophagitis (EoE). A study with prolonged esophageal pH/manometry.

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The pathophysiology of dysphagia in patients with EoE is unknown, but may be related to abnormal esophageal motility function. Stationary esophageal manometry is generally normal, and symptoms rarely occur during its performance, so it is difficult to establish an association between symptoms and motor events.

Methods: A 24 hour pH/manometry was performed in children with EoE (distinctive histopathology, normal pH probe), compared with controls (no dysphagia, niormal ph Probe and esophageal histology) and children with GERD (abnormal pH probe with or without esophagitis). We used a solid state probe with 4 pressure and 1pH transducers that was left 3 cm above the LES. Effective peristalsis = normal amplitude and complete peristalsis. Results are expressed as mean ± SE. ($^*p < 0.05$). **Results:** In EoE the % ineffective peristalsis was the same between mild (74.6 ± 7%), moderate (70.1 ± 7.4%), or severe esophagitis (78.9 ± 8.4%). Thirteen EoE patients had 25 symptoms during the prolonged manometry. All were correlated with abnormal motor function. **Conclusions:** Patients with EoE have ineffective peristalsis during prolonged pH manometry. Therefore symptoms may be related to esophageal motor dysfunction.

Results:	EoE	GERD	Controls
Number of patients	17	13	11
Age (years)	9.7 ± 1.1	13.5 ± 1.3	12.4 ± 1.5
% total time with pH < 4	1.6 ± 0.4	12.4 ± 1.8	3.9 ± 1.4*
LES pressure mmHg	23.7 ± 7.0	24.2 ± 2.11	19 ± 6.2
Median contraction amplitude	57.3 ± 4.5	59.1 ± 5.3	57.3 ± 2.5
% Contractions with amplitude > 25 mmHg	68.2 ± 3.4	72.8 ± 2.9	76.9 ± 2.6
% Contractions with amplitude > 180 mmHg	4.1 ± 1.2	1.8 ± 0.8	0.2 ± 0.1*
% Peristaltic contractions	67.7 ± 3.4	76.3 ±2.2	77.3 ± 2.2
% Total ineffective peristalsis	74.3 ± 4.2	51 ± 6.8	49.6 ± 2.5*
% Ineffective peristalsis after a meal	68.0 ± 4.9	53 ± 4.8	48.1 ± 4.2*
% Isolated contractions	16.7 ± 3.8	9.5 ± 1.6	6.5 ± 1.1*

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Spectral analysis of baseline heart rate variability in children with functional abdominal pain

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Background: The autonomic nervous system (ANS) is viewed as a part of the integrated brain - gut axis. Alterations in ANS activity have been found in adults with abdominal pain and children with

psychosomatic symptoms and anxiety. Heart rate variability (HRV) is a noninvasive index of spontaneous changes in ANS activity and can be evaluated via power spectral analysis of the ECG signal.**Aims:** We investigated differences in baseline HRV indices in pediatric patients with functional abdominal pain (FAP) and well children using two types of spectral analysis: Fast Fourier Transformation (FFT) and wavelet analysis. **Materials and methods:** Subjects were 8 -16 years of age (M = 12.3) (n=47 FAP; n = 22 well). Five minutes of baseline (sitting, awake) heart rate was continuously recorded on a dual-lead ECG system (Biopac Inc.). HRV parameters were calculated directly from electrode tracings after customized peak detection (HRVAnalyzer©, Diedrich). Data were analyzed using FFT and wavelet techniques. Several frequency domains were produced: total 5 min power (TP); high frequency (HF) 0.15-0.4 Hz; low frequency (LF) 0.04-0.15 Hz; and very low frequency (VLF) ≤ 0.04Hz. LF and HF measures were used to calculate sympathovagal balance (SVB), a ratio of LF/HF to reflect ANS modulations on HRV. Time domain measures of HRV also were evaluated. We used 2 sample t-test for parametric and Kruskal-Wallis for non-parametric continuous outcome measures, linear regression for age related outcomes, and a mixed effects model to evaluate group differences. **Results and Conclusion:** FFT and wavelet analysis did not differ significantly on time or frequency domain indices; therefore either technique is appropriate when analyzing 5 minute HRV. FAP patients differed from controls in the LF/HF ratio, the LF and HF correlation, and other frequency domain indices, suggesting that atypical baseline characteristics may be present in FAP children. Most HRV indices were age dependent at $p < .05$. Because of developmental differences in maturation of ANS, age-matching of subjects should be considered in pediatric studies. The positive linear correlation between LF and HF is consistent with findings from previous pediatric studies. Further study of differences in HRV variability indices between FAP and well children is warranted.

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Interstitial Cells of Cajal in pediatric gastrointestinal motility disorders

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Background: Interstitial Cells of Cajal (ICC) are considered the pacemaker cells of the enteric nervous system. The decrease in number and/or absence of those cells as well as their quality have been linked to dysmotility of the gastrointestinal tract. Little is known about their role in clinical dysmotility syndromes in pediatrics. We present preliminary data of a prospective study evaluating the role of Interstitial Cells of Cajal in gastrointestinal motility disorders in the pediatric population. We also include preliminary data on neurotransmitter levels in tissue biopsies. **Methods:** Patients evaluated for motility disorders including chronic intestinal pseudo-obstruction, severe colonic dysmotility requiring ileostomy and internal anal sphincter achalasia that undergo clinically indicated surgical procedures were included and full thickness intestinal, colonic and/or rectal biopsy specimens were evaluated. The presence and quality of ICC is evaluated, and when tissue is available and properly collected neurotransmitter levels in tissue are evaluated including CGRP, Galanin, Substance P, VIP, NPY and PYY as well as NOS, (neuromodulator) and S100 and Pgp9.5 (neural markers). **Results:** 16 patients have been evaluated, 8 with internal anal sphincter achalasia, 2 with chronic intestinal pseudo-obstruction and 6 with colonic dysmotility. Of the 5 patients with colonic dysmotility, 2 had decreased or absent ICC in the sigmoid colon (normal in small bowel) and another had decreased neurotransmitters. Of the 2 patients with chronic intestinal pseudo-obstruction, both had normal ICC in small bowel and one had absent ICC in sigmoid colon, neurotransmitter studies were not performed. Of the 8 patients with internal anal sphincter achalasia, 3 had decreased ICC and one other had absent ICC in the rectum, only 4 underwent neurotransmitter studies and were all normal. **Conclusions:** ICC's play an important role in gastrointestinal motility disorders in children and adolescents. Further studies are needed to understand their role in clinical practice.

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Long-term outcomes of pediatric chronic abdominal pain

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Children with chronic abdominal pain (CAP) are thought to be at risk for maintenance of abdominal pain and development of additional chronic pain and emotional disorders as adolescents and young adults. We reviewed the literature on the long-term outcomes of CAP to assess what is known, the methodological strengths and weaknesses of the literature, and gaps in the literature.

Online bibliographic databases were searched for the terms "recurrent abdominal pain" or "chronic abdominal pain'' in articles from 1950 and later. Inclusion criteria included the following: at least 10 participants with CAP or RAP (definitions were allowed to vary), younger than 18 years of age at baseline, outcomes assessed at least 5 years following baseline, and the study was not a clinical trial. Ten studies met these criteria. The methodology and findings of these articles were evaluated critically, and data were extracted from each article regarding study design and setting, study methods, outcomes measured, and results.

Of the 10 studies, 2 used community samples and 8 were conducted in a clinical setting and involved a medical evaluation at baseline. Baseline assessment was prior to 1975 in 50% of the studies. The time interval between baseline and outcome assessment ranged from 5 years to 36 years. A retrospective design was employed in most studies (80%). Inclusion/exclusion criteria and medical evaluation varied greatly across studies. Although most studies (70%) used Apley's criteria for RAP, 3 studies used other criteria. Most studies (60%) did not include a well control group. Validated questionnaires were used to assess outcomes in only 40% of the studies. Studies varied greatly in the types of outcomes assessed, making comparison of results difficult. Maintenance of abdominal pain at follow-up ranged from 24% to 53%. Functional outcomes and psychiatric diagnoses were rarely assessed.

Despite the common assumption that children with CAP are at risk for long-term maintenance of CAP and development of other pain conditions and psychiatric disorders, the evidence base for long-term outcomes of CAP is weak. Prospective studies are needed to specify outcomes and identify those characteristics that predict long-term maintenance of CAP and development of other conditions.

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Synchronized gastric electrical stimulation improves gastric emptying in non-obese mice with diabetic gastroparesis

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Background: Synchronized gastric electrical stimulation (SGES) has been reported to enhance antral and small intestinal contractions in both fasting and fed states. However, it is unknown whether SGES could improve gastric motility in a murine model of diabetic gastroparesis (D-GP). **Aims:** The aim of this study was to investigate the effect and mechanism of SGES on gastric emptying in non-obese mice with diabetic gastroparesis. **Methods:** Eight control mice and 40 non-obese diabetic (NOD) mice with two pair of electrodes were used in this study. The study included six groups in a randomized order (control, Diabetes (DB), D-GP, DB+SGES, D-GP+SGES, and D-GP+SGES+Atropine groups). In the control, DB or D-GP group, gastric emptying was measured by the assessment of gastric retention (30min after the meal) of phenol red mixed with 0.2 ml of methylcellulose gavage fed in BLAB/cJ mice (control group) or NOD mice with a duration of diabetes of 0-7 days (DB group) or 28-35 days (D-GP group). In the DB+SGES or D-GP+SGES group, the experiment was the same as the DB or D-GP group except that SGES was applied during the experiment. In the D-GP+SGES+Atropine group, the experiment was the same as D-GP+SGES group except the injection of atropine. SGES was applied via the proximal pair of electrodes and synchronized with the intrinsic gastric slow waves. The stimulus was composed of trains of pulses with a frequency of 40Hz, pulse width of 2ms and amplitude 4mA. **Results:** Gastric emptying was delayed in NOD mice with a duration of diabetes of 28-35 days. Gastric emptying was 78.7±5.9% in

the controls, 59.0±6.3%, (P<0.01 vs controls) in the D-GP and 77.6±10.2% (P=0.72 vs controls) in the DB group. 2) SGES was able to significantly increase gastric emptying in both diabetic mice and diabetic gastroparetic mice. Compared with that in DB or D-GP group, gastric emptying was significantly enhanced in DB+SGES (85.2±4.9%, P=0.017) and D-GP+SGES (72.8±11.6%, P=0.016) groups. 3) The excitatory effect of SGES was completely blocked by atropine. In contrast to that in the D-GP group, gastric emptying in D-GP+SGES+Atropine group was not significantly improved (57.3±10.1%, P>0.05).**Conclusions:** SGES accelerates gastric emptying in NOD mice with diabetic gastroparesis. The effect of SGES on gastric emptying is mediated via the cholinergic pathway. These findings suggest that SGES may have a therapeutic potential for treating patients with gastroparesis (Supported by a grant from American Diabetes Association).

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Inter-observer variability in interpretation of colon motility studies in children

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Introduction: A conventional colon motility (CM) study for evaluation of chronic constipation takes about 5-6 hours to perform and requires fasting, post-prandial and often bisacodyl stimulation to induce high amplitude propagating contractions. The need for post-prandial recording is to document a gastrocolonic response. Using a standardized meal in children is difficult and often patients refuse to eat the required amount, therefore the significance of a gastrocolonic response has been questioned in pediatric studies. **Aims:** To compare inter-observer variability in interpretation of colon manometry and to evaluate if postprandial recording helps in clinical decision making. **Method:** Three pediatric gastroenterologists, blinded to the patient identity and diagnosis, evaluated 38 CM studies and also commented if postprandial recording helped their clinical decision making. The diagnosis and interpretation of each clinician was compared with other two. We calculated crude agreement i.e. percentage agreement not corrected for chance and to account for possibility of agreement by chance we calculated the kappa coefficient (k), a measure of reliability for categorical data. **Results:** The data are expressed as the percentage agreement between clinicians for the fasting, post prandial, post bisacodyl and overall study result being either normal or abnormal (see table). The kappa values are shown in parenthesis. The * indicates the p value <0.0001, the probability that the agreement between clinician was not by chance. In more than 90% of the studies the clinicians agreed that the postprandial recording was not helpful in clinical decision making. **Conclusions:** 1) Fasting and post bisacodyl recording provides sufficient information for clinical decision making in >90% of children. 2) The agreement between the three clinician's regarding gastrocolonic response and post prandial abnormalities was poor. 3) Further studies to evaluate which patients would require a full CM study and which can have shortened study including 30 minutes of fasting followed by bisacodyl stimulation are required.

	Clinician 1 & 2	Clinician 2 & 3	Clinician 2 & 3
Fasting period	100%	100%	100%
Post-prandial	42% (0 .44)	29% (0.24)	50% (0.39)
Response to Bisacodyl	92% (0.87)*	94% (0.88)*	94% (0.87)*
Overall study result	87% (0.94)*	81% (0.76)*	82% (0.81)*

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Aerophagia induced eructation as detected by 24 hour pH and impedance testing

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Prolonged pH monitoring for 24 hours has been considered the gold standard for measuring gastroesophageal reflux. In recent years the technology has enabled the use of impedance monitoring which can measure the nonacid reflux material in the esophagus. The technique also measures gas, liquid, mixed gas-liquid material moving in the esophagus.

Patients who are compulsively air swallowing are sometimes unaware that this is occurring. This aerophagia can cause reflux of acidic material that can become pathologic in frequency, and cause mucosal damage to the esophagus. This method not only shows that there is aerophagia induced acid and nonacid reflux, it only occurred during waking hours, not during sleep.

With the patient recorded diary information it is possible to see the number of swallows, the number of eructations and the pathologic amount of reflux associated with the aerophagia induced eructation. Symptoms correlate on the tracing with the events recorded for acid and nonacid reflux.

Evaluation of aerophagia induced pathological reflux is a novel use of esophageal pH and impedance monitoring. The frequency and severity of the problem can be quantified by pH impedance testing.

Review of the literature showed use of this technique in Genoa, Italy in a single case of an adult woman with aerophagia. The only other reference to this application is from the Netherlands comparing 14 patients with excessive belching to 14 healthy controls. The youngest patient was 18 years of age. The patients showed a pattern of supragastric eructations of swallowed air from the esophagus without increased gaseous reflux from the stomach.

By contrast, we see this technique as having value in the pediatric age ranges. The first patient studies we have done have also shown that pathologic amounts of gastroesophageal reflux are associated with aerophagia induced eructation.

This information is helpful in making the diagnosis and in making the treatment plan for the patient. There have been few tools available to physicians for evaluating functional gastrointestinal problems, and this application may represent an advance in being able to document the physiologic activity causing the problem. Progress can be monitored by a follow-up pH impedance study as well.

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Do oro-anal transit marker studies correlate with colonic manometry studies in children?

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Introduction: A majority of children with constipation have a functional disorder and get better with medical therapy. In one study, 33% children with chronic constipation were reported to have slow oro-anal transit time (OTT). Colon neuromuscular abnormalities, assessed using colon manometry (CM), have also been reported in children with chronic intractable constipation. To date, no pediatric studies have compared the OTT with CM findings in children with chronic constipation. **Aims:** To compare OTT measured by radio-opaque markers with CM findings. **Methods:** The records of 10 children with chronic constipation [6 females; median age 12y (5-18y); median symptoms 73 mo (6-176 mo)] who underwent OTT and colon motility studies were reviewed. Normal colonic transit was defined as retention of 8 or fewer radio-opaque markers in the left or recto-sigmoid colon at 72 hours using a modified Bautista method. CM study was performed using an 8 channel water-perfused system and included continuous recordings of fasting baseline, stimulated (bisacodyl 5-10 mg) and post-prandial segments of 1 hour each. Normal CM was defined by the presence of HAPCs (colonic contraction amplitudes of at least 60 mmHg propagating in an aboral pattern over at least 30 cm bowel). Normal gastro-colonic (GC) response was defined as a >30% increase in contraction area under the curve between fasting and post-prandial segments. **Results:** OTT marker studies were 100% sensitive and 67% specific for CM abnormalities. **Conclusions:** Normal OTT studies may predict normal colon manometry. However, abnormal OTT studies may not predict abnormalities in colonic manometry or GC response in children with chronic constipation. Therefore, patients with slow OTT marker studies should be assessed by colon manometry to evaluate

colon neuromuscular integrity. Prospective studies are warranted to confirm these findings.

OTT	Spontaneous HAPCs		Stimulated HAPCs		GC Response		Overall Manometry	
	Absent	Present	Absent	Present	Abnormal	Normal	Abnormal	Normal
Slow (n=6)	6	0	3	3	4	2	4	2
Normal (n=4)	2	2	0	4	1	3	0	4

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Healthcare utilization before and after biofeedback for childhood recurrent abdominal pain

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Background: Childhood recurrent abdominal pain (RAP) is associated with increased healthcare utilization. Therapeutic biofeedback (BF) attempts to achieve behavior modification toward pain and has been used in the treatment of RAP. A decrease in healthcare utilization would serve as a valid endpoint, marking efficacy of this therapeutic intervention in RAP. **Objective:** To determine healthcare utilization of children with RAP after completing biofeedback. **Subjects & Methods:** A database of a large health maintenance organization was queried to identify, all children from 2003—2005 who were diagnosed with RAP by a pediatric gastroenterologist and who then completed BF treatment. The data were collected over the 2-year period prior to BF treatment and 1-year post treatment. Outcome measures used included: total number of health care visits, prescriptions, emergency room visits, and diagnostic evaluations. **Results:** 39 children (55% female, aged 13.4 years, range 8 to 19 years) had been diagnosed with RAP and undergone BF treatments (4.9 sessions, range 1-12). **Conclusions:** In this cohort of children with RAP who were treated with biofeedback, there was a marked decrease in healthcare utilization. Biofeedback should be considered as a cost effective first line therapy for childhood RAP. Long-term studies need to be performed to see if this positive outcome effect persists into adulthood.

	BEFORE BF	AFTER BF	SIGNIFICANCE
Primary Care Visits	10.3	3.8	P < 0.001
Emergency Room Visits	0.69	.05	P < 0.001
Prescriptions	8.8	4.5	P < 0.004
Diagnostic Tests	0.77	.08	P < 0.001

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Treatment for childhood recurrent abdominal pain: how are they doing two years later?

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Background: Children with recurrent abdominal pain (RAP) have increased rates of healthcare utilization and are at risk for psychiatric co-morbidities as adults. **Aim:** To compare treatments for RAP and document gastrointestinal (GI) symptoms, health care seeking and development of co-morbidities 2 years after treatment. **Subjects/ Methods:** From 06/ 2002 to 6/ 2003, 96 children (mean age, 11.1 years, range 6-18 years, 55% female) with RAP who had undergone diagnostic evaluation by pediatric gastroenterology and treated with either low dose tricyclic antidepressants (LDTCA, n= 40) or standard of care: education and reassurance (EAR, n=56) were identified. Chart review for demographics, duration of medical evaluation, treatment, and subsequent development of organic GI disease was performed. A 15-item telephone survey was conducted 2 years after treatment. Outcomes included: 1) Does your child have abdominal pain > 3 times weekly, 2) Is your child on any medications (antacids or laxatives) for GI symptoms, 3) Have you sought consult from other specialists (rheumatology, neurology, infectious disease) for your child, and 4) Is your child being treated for anxiety or depression. Age and gender matched children without abdominal pain undergoing routine physicals in 2003 served as healthy controls (HC, n=20). **Results:** At 2 years: 46 patients (48%) have abdominal pain > 3 times /week after treatment. Differences between LDTCA and EAR were found regarding persistent abdominal pain (65% vs. 33%, $p<0.05$) and need for GI medications (45% vs. 14%, $p<0.05$). None were on antidepressants for abdominal pain. No patient had organic GI disease. No difference in encounter time with gastroenterology during treatment: (LDTCA, 3.9 ± 3.1 months vs. 3.5 ± 3.7 months for EAR, p=ns). No HC developed RAP. No patients had consult with other specialists. One patient in HC developed anxiety (5%). No differences for anxiety/depression in LDTCA vs. EAR (7% vs. 8%, p=ns). **Conclusions:** In this cohort, nearly 50% of all children with RAP continue to have pain 2 years after treatment. Those with ongoing RAP and need for GI medications were more likely to have been treated with LDTCA. Longitudinal studies on children with RAP after LDTCA treatment need to be performed to see if they become those adults that suffer from both IBS and somatization disorder, often the most difficult to treat. Identification of these subsets of RAP patients in childhood may allow for other interventions such as coping strategies to be offered earlier.

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The presence of overlap syndromes in patients with gastroparesis and correlation with hypercoagulable states in gastroparesis

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Introduction: Many patients with gastroparesis (GP) have overlap syndromes such as migraine headaches, fibromyalgia, endometriosis, and interstitial cystitis, all of which may be immune mediated. We have previously shown (GE 108: A734, 1995) that hypercoagulability in GP is associated with serological evidence of autoimmunity, which frequently results in vascular complications of therapies for GP. Thus, we hypothesized that overlap syndromes may be related to hypercoagulability in GP. **Methods:** We studied 76 consecutive patients (65 female, 11 male) with a mean age of 43 years who all met clinical criteria for gastroparesis. The patients, evaluated for possible gastrointestinal electrical stimulation, underwent medical evaluation including a standardized history to detect the presence of overlap syndromes and laboratory measurements for both acquired and congenital defects of coagulation: Factor VII, Factor VIII, Fibrinogen, Antiphospholipid Antibodies, Activated Protein C Resistance (APCR), Lupus Anticoagulant, Methlenetetrahydrofolate resistance (MTHFR), and Factor II Mutation. Patients were also stratified into groups based on which of the overlap disorders were present. Patients with a diagnosis of depression were also stratified based on the overlap disorders. **Results:** All patients had gastroparesis symptoms and 70/76 (92%) of the patients were found to have at least one of four overlap disorders: migraine headaches, interstitial cystitis, endometriosis, and fibromyalgia. There were 42/76 (55%) of the patients with a diagnosis of depression, and 34/76 (44%) of the patients had more than one overlap disorder. Also, 60/76 (78%) of the patients were found to have defects in coagulation in addition to overlap disorders. **Conclusion:** We conclude that overlap disorders are found frequently in patients with drug refractory gastroparesis and may be related to hypercoagulable states through a shared mechanism of altered immunity. These patients offer the opportunity to investigate specific pathophysiology. Therefore, it may be useful to screen gastroparesis patients for overlap disorders in order to maximize therapy. Also, patients with overlap disorders and gastroparesis frequently have depression regardless of the number of overlap disorders present. In addition, assessing patients with gastroparesis who present with overlap disorders for hypercoagulable states may affect patient management, and thus minimize potential complications of therapy.

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Vaccination of IBS patients after unsuccessful antibacterial treatment

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Background We have successfully used antibacterial treatment for IBS patients, but in some of them relapses occurred and we had to use repeated antibacterial treatment for two or three times. Since further treatment with antibiotics could be dangerous and irrational and we believed that these relapses were due to reinfection or immune tolerance to the offending microorganisms, we decided to use a vaccine containing the most prevalent facultative pathogens to boost immune response in these patients. **Materials and methods** We used standard vaccine created for treatment of patients with urinary tract infections (Solco-Urovac, Swizerland), which contains most frequently encountered faecal facultative pathogens usually causing urinary tract infections. We vaccinated 50 patients meeting Rome criteria for IBS previously unsuccessfully treated with antibacterials two or three times and used placebo (normal saline) in the group of 50 patients meeting the same criteria. Fecal flora was evaluated by culturing enterobacteriacea and paying attention to facultative pathogens, before and 2-3 weeks after vaccination and for all patients with worsening of symptoms, not more than 6 months later. **Results** Colonization by facultative pathogens such as Klebsiella, Proteus, Enterobacter, E. coli abnormal strains, etc. was detected in fecal flora of all patients. Clinical improvement was observed in all vaccinated patients in two weeks after vaccination. 48 patients had relapses (less expressed) after 1 to 6 months. 14 patients had improvement of symptoms without improvement in microecology. Clinical improvement was observed in 6 patients out of 50, who received placebo. **Conclusions** The changes in the colonic flora of IBS patients might be due to weak or exhausted immune response against facultative microorganisms causing IBS symptoms. IBS patients could be successfully treated using vaccination. Insufficient efficacy might be due to incomplete match between antigens of the offending microorganisms and vaccine. The use of autovaccine could be more effective.

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Rapid Activation of Early Growth Response gene-1 (Egr-1) after Surgical Manipulation of the Intestine

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Introduction: Postoperative ileus is caused by the initiation of a complex molecular and cellular inflammatory response within the intestinal muscularis. The transcription factor, early growth response gene-1 (Egr-1), is known to interact with a zinc finger DNA-binding site on many pro-inflammatory genes. Our objective was to examine the generation of Egr-1 protein and determine its functional influence on jejunal circular smooth muscle contractility following surgical manipulation of the small intestine. **Methods & Results:** Laparotomy and surgical manipulation of the murine intestine resulted in the rapid, time-dependent generation of Egr-1 protein, as determined by Western blot with fold increases at 30, 90 and 180min measuring 13.1±1.92, 22.7±2.84 and 17.6±2.74, respectively (N=3). And at 12 and 24hrs only trace protein amounts were evident demonstrating a return to the undetectable amounts measured in control tissue extracts. These data correspond to a 55.4±9.89 fold induction in Egr-1 mRNA at 3 hrs. Jejunal circular muscle function in response to bethanecol was evaluated using a mechanical organ bath setup. Bethanechol caused a dose-dependent increase in muscle contractility which was similar between Egr-$1^{+/+}$ and Egr-$1^{-/-}$ mice (104±17.5% of control at 100 μM bethanechol). Surgical manipulation resulted in a suppression in muscle contractility harvested from Egr-$1^{+/+}$ mice (55±3.8 of control response), but this suppression was significantly less evident in the Egr-$1^{-/-}$ mice, which had contractile responses at 100 μM bethanechol similar to unmanipulated mice (95±12.5 of control response) (N=6 each). **Conclusion:** These findings indicate that Egr-1 plays a significant role in the inflammatory response of postoperative ileus and that inhibition of this transcription factor may be a novel pharmacological target to avert postoperative ileus.

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Autoantibodies in sera from patients with type 2 diabetes and neuropathy induce autophagy via activation of Fas in human neuroblastoma cells

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Background: We reported previously that sera from patients with type 2 diabetes and neuropathy (D+N) induce apoptosis and activate macroautophagy (autophagy), a putative cytoprotective pathway, in human neuroblastoma (SH-SY5Y) neurons. Autophagosomes contain damaged proteins and organelles, including mitochondria. Agonist and antagonist autoantibodies directed against Fas (CD95) have been reported in health and disease. We hypothesized that autoantibodies present in sera from D+N patients would activate autophagy via a Fas-dependent pathway. **Methods:** SY5Y cells were incubated overnight with complement-inactivated sera (1:10-1:320 dilution) from normal (N; n=6), D+N (n=8) and type 2 diabetic patients without neuropathy D–N (n=4). **Results:** D+N sera but not D-N or control sera differentially activated autophagy, monitored using the specific autophagosome marker microtubule-associated protein 1 light chain 3-II, (LC3-II), by Western blot (WB) and immunohistochemistry (IHC) in a titer-dependent manner, and colocalization with mitochondria using anti-mitochondrial antibody (AMA). D+N sera significantly increased Fas mRNA and protein, Fas-activated Death Domain (FADD) mRNA and protein and colocalization of anti-LC3 with anti-FADD using IHC. Gene silencing using si-FADD significantly decreased the effect of D+N sera on levels of FADD mRNA and protein, and the induction of the autophagosomes. Pre-incubation of D+N sera with soluble Fas receptor (extracellular domain) significantly decreased binding of autoantibodies to SY5Y cells, and markedly reduced induction of Fas/FADD and formation of autophagosomes. Enrichment of IgM and IgG fractions using sucrose gradient ultracentrifugation revealed that the IgM fraction preferentially activated autophagy. **Summary/Conclusion:** These results support the novel and provocative observation that increased levels of autoantibodies (IgM>IgG) present in sera of type 2 D+N patients bind Fas and activate formation of autophagosomes and colocalization of mitochondria and FADD.

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Temporal relationship between changes in c-Kit, nNOS and HO1 expression in early diabetes

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Background: Established defects in diabetic gastroparesis include loss of expression of nitric oxide synthase (NOS) in enteric neurons and loss of interstitial cells of Cajal (ICC). Recent work has highlighted a potential role for NO, derived from nNOS, as a cytoprotective factor for ICC suggesting the possibility that loss of ICC in diabetic gastroparesis is a result of loss of nNOS. **Aims:** Determine the temporal relationship between onset of diabetes and expression of nNOS, c-Kit, expressed in ICC, and HO-1, which generates potent antioxidant and anti-apoptotic molecules. **Methods:** nNOS, c-Kit and HO-1 protein expression was determined by quantification of Western blots using protein extracted from the gastric body of diabetic NOD mice. Mice were divided into three groups. Group 1 (n=6) were non diabetic NOR or NOD mice age matched to Groups 2 and 3. Group 2 (n=6) were NOD mice, diabetic (>250 mg/dl) for 1-2 weeks and Group 3 (n=4) were NOD mice diabetic (>250 mg/dl) for 4-5 weeks. Gastric emptying was determined by the [^{13}C]-octanoic acid breath test. **Results:** None of the mice had delayed gastric emptying. Mean $T_{1/2}$ (±SEM) for Group 1 was 109±10 min. $T_{1/2}$ for Group 2 was 58±4 min and 68 ± 7 min for Group 3. Median (inter-quartile ranges) blood glucose level was 105 (97-112) mg/dl in Group 1, 480 (361-585) mg/dl in Group 2 and 500 (494-525) mg/dl in Group 3. c-Kit expression was similar in all three groups (99 ± 3.8% and 104 ± 21%, of controls for Groups 2 and 3 respectively, P>0.05). nNOS expression was not decreased in Group 2 (74 ± 20%, P>0.05) but was significantly decreased in Group 3 (65 ±

12%, P<0.05) compared to Group 1. HO-1 expression was unchanged in Group 2 (74 ± 14%, P>0.05) and increased in Group 3 (150 ± 20% P<0.05) compared to Group 1.**Conclusion:** In early diabetic NOD mice, nNOS expression is decreased and HO-1 expression increased. These changes occur prior to loss of c-Kit expression and before onset of delayed gastric emptying. These data, taken together with previous data suggesting a cytoprotective effect of NO on ICC, indicate that the sequence of events in the development of diabetic gastroparesis includes early loss of nNOS followed by loss of ICC. It remains to be established whether the increase in HO-1 expression 4-5 weeks after onset of diabetes has a cytoprotective effect. This work is supported by NIH grants DK68055 and DK57061.

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Ultrastructural evidence of apoptosis in interstitial cells of Cajal from the colon of patients with slow transit constipation

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Background: Loss of interstitial cells of Cajal (ICC) or disrupted networks of these cells are associated with several motility disorders including slow transit constipation. However, the mechanism of ICC loss is unknown. We have previously reported increased levels of TUNEL and activated caspase-3 in ICC from the colon of patients with slow transit constipation and also observed evidence of apoptotic ICC in the normal colon. **Aims:** To confirm that ICC die by apoptosis in slow transit constipation and that apoptosis also occurs in ICC in normal colon by studying changes in the ultrastructural morphology of ICC. **Methods:** Tissues from the right and left colon of 2 female patients with well defined slow transit constipation and control tissues from patients undergoing colectomy for cancer were used to investigate whether apoptotic ICC could be identified by electron microscopy. The patients with slow transit constipation were a 24 year old with a 5-8 year history of constipation and a 28 year old with a 20 year history of constipation. Colon tissues were fixed in 2% glutaraldehyde in 0.1M cacodylate buffer and processed for electron microscopy. **Results:** ICC in the muscle layers and myenteric plexus regions of tissues from the patients with slow transit constipation had cellular damage consistent with apoptosis. These ICC had evidence of condensed chromatin, swollen mitochondria and dilation of the perinuclear space. ICC with other markers of pre-apoptotic injury including a clear cytoplasm and vacuolation of the cytoplasm were also observed. In tissues from control patients, ICC with morphological changes consistent with apoptosis were also detected. However apoptotic ICC were qualitatively more common in tissues from patients with slow transit constipation. **Conclusion:** Electron microscopy data confirm that apoptosis occurs in ICC in both normal human colon tissue and in colons of patients with slow transit constipation. We conclude that apoptosis contributes to the depletion of ICC in slow transit constipation and that there must be turnover of ICC in the normal human colon to replenish apoptotic cells and maintain intact ICC networks. Supported by NIH DK52766 and DK57061 and MURST from Italy.

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Substance P mediated excitation masks inhibitory innervation in the WW^v mouse fundus

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Studies into the role of ICC-IM in innervation have produced controversial results in different species and organs. The objective of the present study was to re-examine this role in the mouse fundus, since studies in this tissue initiated the hypothesis that ICC were essential in inhibitory innervation. The response of muscle contractile activity to field stimulation of enteric nerves was studied. In wild type mice, the fundic musculature underwent inhibition of contractile activity when enteric nerves were stimulated in the presence of atropine and guanethidine. Quantitatively, nerve stimulation in wild type mice reduced muscle strip tone from 21.9 ± 3.2 to 15.8 ± 4.1 (mN/mm^2).min (P< 0.01)(n=10). When the nerve stimulation was repeated in the presence of L-NNA, the contractile activity increased from 15.8 ± 2.1 to 25.0 ± 5.1 (mN/mm^2).min (P< 0.01). Subsequent studies revealed that a large part of neurally mediated contractile activity was mediated by non-cholinergic enteric excitatory nerves. In WW^v mice, fundic contractile activity before and after electrical stimulation in the presence of atropine and guanethidine was 24.4 ± 3.2 and 22.7 ± 3.1 (mN/mm^2).min (n=12) respectively; When the nerve stimulation was repeated in the presence of L-NNA, WW^v fundic muscle preparations that showed relaxation before addition of L-NNA, showed contraction after addition of L-NNA. Other preparations showed increased contractile activity. The values were 19.1 ± 2.5 before and 23.5 ± 3.2 (mN/mm^2).min during nerve stimulation (P<0.05). The NO mediated relaxation was normal in WW^v mice. The non-cholinergic excitation, was inhibited by the NK1 antagonist SR140333 (10^{-7}M) indicating the presence of substance P mediated excitation in both wild type and WW^v tissue. To confirm SP mediated excitation, nerve stimulation in the presence of atropine, L-NAME and apamin caused contractile activity that was inhibited 51.4 ± 11% (n=7) by the NK1 antagonist SK14033 in WW^v mice, whereas it was inhibited only 8.7 ± 0.2% in control mice. Hence, in WW^v mice the absence of ICC resulted in stronger substance P mediated excitation. Hence, either neurokinergic innervation is increased in WWv mice or SP nerves innervate ICC preferentially and loss of ICC causes direct muscle excitation. During field stimulation of enteric nerves, neurokinergic excitation masks inhibitory innervation in WW^v mouse fundus. Supported by CIHR and CAG.

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ICC protect smooth muscle from excessive excitation

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The role of ICC-IM in innervation of the stomach was investigated using the Ws/Ws and wild type rat stomach. Ultrastructural, mechanical and electrophysiological investigations were performed. Ultrastructural studies revealed synapse like contacts from enteric nerves to ICC and to smooth muscle cells, providing the structural evidence for direct muscle innervation. Electrical stimulation of enteric inhibitory nerves produced marked decrease in muscle tone of wild type but not Ws/Ws rats suggesting impairment of inhibitory innervation consistent with the literature. However, when effects of nerve stimulation was studied on the marked phasic contractile activity that developed in the Ws/Ws rat fundus, NO mediated inhibition was 2.0 ± 0.2 (mN/mm^2).min in wild type and 3.2 ± 0.4 (mN/mm^2).min in the WsWs rat fundus (n=10), not different. The addition of L-NAME to inhibit NO synthesis and apamin revealed marked non-cholinergic excitation which was significantly larger in Ws/Ws fundic muscle compared to wild type (n=10). Electrical stimulation of enteric nerves in the presence of atropine and guanethidine evoked inhibitory junction potentials (ijp's) consisting of a fast and slow component in both wild type and Ws/Ws fundic muscle cells. A NO mediated ijp of 8 ± 2 s duration in wild type and 10 ± 3 s duration in Ws rats (n=6) indicated that nitrergic nerves could innervate smooth muscle cells in Ws/Ws rats. The apamin sensitive inhibitory innervation likely mediated by ATP, was normal in Ws rats according to mechanical and electrical assessments. In vitro measurements of gastric relaxation of the whole stomach in response to distention were carried out in the wild type and WW^v mice. Total tension development in response to distention (1 ml over 10 min) was markedly affected by blockage of NO synthesis whereas adaptive relaxation was primarily mediated by apamin sensitive inhibitory nerves. In conclusion, in the Ws/Ws rat, NO mediated inhibitory innervation can only be revealed by studying phasic contractile activity changes in light of a low basal tone, inhibitory innervation is present to allow for accommodation and nerve stimulation evokes marked non-cholinergic excitation in the absence of ICC-IM. Supported by CIHR and CAG.

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Use of *Ws/Ws* mutant rats to investigate the role of ICC in pacemaker activity and enteric motor transmission in the stomach and small intestine

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Mutant animal models have been exploited in recent years to elucidate the functional roles of ICC. For example, the W/W^V Kit mouse has been used to identify a role of ICC (ICC-MY) as pacemakers in the small intestine and intramuscular ICC (ICC-IM) as mediators for enteric neurotransmission in the stomach. Although murine models are useful there are often limitations in size and tissue amounts that preclude the use of these animals in many experimental procedures. The *Ws/Ws* mutant rat also has loss of specific populations of ICC throughout their GI tracts and these animals may be more useful for certain experimental procedures. The loss of ICC in *Ws/Ws* rats has been described using Kit immunohistochemistry and electron microscopy but quantitative functional studies have yet to be performed. The aims of the present study were to examine the role of ICC in pacemaking and neurotransmission in the stomach and small intestines of *Ws/Ws* rats using intracellular recording techniques. In the gastric fundus of wildtype rats, RMP averaged -45 mV and slow waves were absent. Stimulation of enteric nerves produced frequency-dependent IJP's (0.3-0.5 ms, 1-20 Hz for 1 s) that were sensitive to L-NNA (100 μM) and to PPADS (30 μM). In the gastric antrum RMP were −65 mV and slow waves 25 mV in amplitude occurred at a frequency of 6 cycles min^{-1}. Electric field stimulation (EFS) of enteric nerves produced large IJP's and reduced the amplitude of slow waves. In the small intestine of wildtype rats RMP averaged −68 mV and slow waves 30 mV in amplitude occurred at a frequency of 25 cycles min^{-1}. In the fundus of *Ws/Ws* rats RMP was −55 mV and slow waves were absent. In the antrum of *Ws/Ws* rats, RMP was −60 mV and smaller slow waves were recorded compared to control. In two *Ws/Ws* animals slow waves were absent. EFS in the fundus and antrum produced IJPs that were not sensitive to L-NNA (100 μM) but was sensitive to PPADS (30 μM). In the small intestines of *Ws/Ws* rats slow waves were absent, but large IJPs were evoked in response to EFS. IJPs in the small intestines of *Ws/Ws* rats were sensitive to L-NNA (100 μM). These data suggest that the activities of *Ws/Ws* rats are similar to W/W^V mutant mice. Nitric oxide dependent neural responses are attenuated in the stomach and are associated with a loss of ICC-IM. Pacemaker activity is absent in the small intestine but neural responses persist. This is associated with a loss of ICC-MY but not ICC-DMP. In conclusion, *Ws/Ws* mutant rats are a useful animal model to investigate the role of ICC in a larger animal model. Supported by NIH DK57236.

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Presence of cells with morphological and physiological characteristics of interstitial cells of Cajal in the guinea pig gallbladder

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Gallbladder smooth muscle (GBSM) cells exhibit spontaneous rhythmic electrical activity and associated Ca^{2+} transients. It is not clear whether the origin and propagation of action potentials and related contractile activities in GBSM involve specialized pacemaker cells identified in the GI as interstitial cells of Cajal (ICC) or are intrinsic properties of GBSM cells. The goal of this study was to determine the presence of ICC in the gallbladder using morphological and physiological approaches. Immunohistochemistry for Kit tyrosine kinase, a specific marker of ICC in the GI tract revealed a population of cells in the gallbladder with characteristics similar to ICC in the GI of the guinea pig. These Kit-positive ICC-like cells were elongated with one or two primary processes emerging from the cell body and aligned with GBSM and nerve fibers. By transmission electron microscopy these cells were rich in mitochondria, caveolae and sER and formed close appositions with GBSM, but not with nerve fibers. Confocal imaging of calcium transient showed that both GBSM and ICC-like cells exhibit spontaneous Ca^{2+} flashes and Ca^{2+} waves. ICC-like cells were of two types i) elongated bipolar cells associated with GBSM cells and ii) multipolar cells located at the origin or intersection of muscle bundles. Ca^{2+} flashes in GBSM and ICC-like cells were synchronized, but whether ICC-like cells are the origin of the activity could not be resolved. Inhibition of Kit tyrosine kinase signaling with imanitib mesylate modulated the generation and propagation of action potentials (APs), Ca^{2+} flashes and Ca^{2+} waves. Imanitib reduced the frequency and disrupted rhythmic pattern of the APs in a concentration- and time-dependent manner, but did not affect membrane resting potential. Imanitib also reduced frequency of Ca^{2+} flashes and Ca^{2+} waves. We tested the role of gap junctions in the propagation of APs and Ca^{2+} flashes in the gallbladder using gap junction uncouplers 1-octanol, carbenoxolone and 18 beta-glycyrrhetinic acid and connexin mimetic peptide P076. Gap junction uncouplers decreased the frequency of APs before abolishing them and depolarized the GBSM cells, abolished Ca^{2+} flashes, reduced Ca^{2+} waves and reduced tissue movement. Connexin P076 decreased the frequency of Ca^{2+} flashes and Ca^{2+} waves. This study provides the first morphological and physiological evidence of ICC-like cells in the guinea pig gallbladder, and our results support a role for these cells in the pacemaking activity in GBSM. (Supported by NIH grant NS 26995).

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In vivo differentiation potential in a heterologous noninjury transplantation model of $Kit^{Low}CD44^+CD34^+$ ICC progenitors isolated from postnatal gastric tunica muscularis

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Recently we have identified $Kit^{Low}CD44^+CD34^+$cells, a rare cell type of the postnatal murine gastric tunica muscularis, as progenitors of interstitial cells of Cajal (ICC) (Gastroenterology 2006;130(4; Suppl 2):A-538). Previously we also reported that cells in a conditionally immortalized line (D2211B) clonally derived from $Kit^{Low}CD44^+CD34^+$cells of juvenile *H-2K*^{b}-tsA58 mice express markers for various cell types including Kit and CD44 (ICC), smooth muscle myosin heavy chain, protein gene product 9.5 (PGP 9.5; neurons), glial fibrillary acidic protein (GFAP), cytokeratin 1-18 (epithelium), CD68 and MHC class II antigens (antigen-presenting cells), but not CD31 (endothelium) or mast cell tryptase (Gastroenterology 2005;128(4; Suppl 2):P-273). In this study we further investigated the stem cell-like characteristics of D2211B cells such as clonality, self-renewal, and in vivo differentiation potential. Fluorescence-activated cell sorting was used to derive secondary subclones from one of the 104 daughter lines of D2211B cells previously created by single-cell sorting. 106 of the 768 single cells harvested grew into clones. RT-PCR analysis of 26 clones indicated the presence of all the cell type-specific markers detected in the original D2211B line, thus verifying the continuing presence of clonogenic progenitors. One of these secondary clones was selected for further studies and found to be capable of dividing beyond 180 population doublings even after inactivating the immortalizing antigen when cultured with suitable media. The in vivo differentiation potential of this line was studied by injecting 20,000-40,000 cells in ovo into the abdominal cavity of E8-10 White Leghorn chick embryos. Sham-injected embryos served as controls. After incubating the embryos at 38°C for 3-8 days their GI tracts were sectioned and immunostained with mouse-specific anti-H-2K^{b}antibodies and antibodies against Kit, smooth muscle myosin, PGP 9.5 and GFAP. Mouse-derived cells were found throughout the GI tracts of the cell-injected embryos. Cells double positive for H-2K^{b}and one of the cell type-specific antigens were detected in the proventriculus, gizzard, and intestines and showed morphological features of subserosal, myenteric and intramuscular ICC, epithelial progenitors and mature epithelium, smooth muscle cells, neurons, and glial fibers. We conclude that ICC progenitors removed from their niche are clonogenic and capable of extensive self-renewal and differentiation into multiple lineages both in vitro and in vivo. Supported by NIH Grant DK58185.

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Interstitial cells of Cajal in the stomach of patients with gastroparesis

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Interstitial Cells of Cajal (ICC) generate electrical pacemaker activity in the stomach. A previous study has shown that ICCs were greatly reduced in the distal stomach of diabetic mice with gastroparesis. The aim of this study was to investigate the status and distribution of ICC in the stomach of patients with gastroparesis. **Methods:** 37 gastroparetic patients (7M, 30F, mean age: 39 years) were studied (23 diabetic, 8 idiopathic and 6 postsurgical). All patients had a full thickness gastric biopsy taken from the antrum and 19 also had a biopsy from the body of the stomach along the greater curvature. These biopsies were obtained in 32 patients at the time of laparotomy for placement of an implantable gastric electrical stimulation system because of lack of response to medical therapy for their gastroparesis. In the other 5 patients a total gastrectomy was performed for refractory gastroparesis (1 diabetic and 4 postsurgical resections) and the entire stomach was available for study. Immunohistochemical staining was performed using the Dako Autostainer (Dako,Carpenteria,CA). Monoclonal antibodies were used according to the standard protocol. The findings were expressed as normal based on scoring system of 20% to 100% intact cells or almost complete loss of ICC to the point that no more than 5 cells were seen per 10 high power fields (less than 10% of the control). **Results:** Based on antral biopsy data, 13 patients (9 diabetic, 3 idiopathic and 1 postsurgical) had no ICC and 24 patients had normal cell numbers. For 19 of 37 patients who had biopsies from both the gastric body and antrum, one had reduced ICC in the body only, two had reduced ICC in the antrum only and 4 had reduced ICC both in the body and antrum. Normal ICCs were seen in 4 of 5 patients with total gastrectomy and Bilroth I or II resections. The other patient with diabetes had depleted ICC in the antrum, but normal ICC in the cardia and body. **Conclusions:** 1) ICCs are absent in a subgroup (35%) of gastroparetic patients who are not responding to standard medical therapy; 2) ICC depletion seems most likely to occur in the antrum, but further studies are needed to understand their distribution in the human stomach in health and disease states.

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The spread of pacemaker activity through a Purkinje-like network of interstitial cells of Cajal in human jejunum

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The small intestine generates rhythmic contractions that are elicited by electrical slow waves originating in a network of pacemaker cells called interstitial cells of Cajal (ICC-My) lying between the longitudinal (LM) and circular (CM) smooth muscle layers. Slow waves propagate into and depolarize the adjacent LM and CM leading to calcium entry and rhythmic contraction. Slow waves are the basis for segmental and peristaltic activity necessary for mixing chyme and absorbing nutrients. Disruption or loss of ICC are associated with motility disorders and compromised peristalsis. To date, calcium fluorescent imaging has been used to study the spread of pacemaker activity through ICC in small mammals that have thin LM and CM layers and a 2-D ICC-My network. **Aims:** Our aims were, therefore, to examine the spread of activity in ICC and LM and CM in human jejunum, that we show to have a more complex 3D network of ICC. **Methods:** Human jejunal segments used in this study were obtained from morbid obesity patients as surgical waste tissues during gastric bypass in accordance with a protocol approved by the University of Nevada, Reno & University of California, Davis Human Subjects Research Committees. A segment of jejunum (10mm x 10mm) was opened and pinned with the serosa uppermost in an organ bath perfused with Krebs' solution at 37°C. Strips of LM were removed to reveal the underlying ICC network. In other experiments preparations were cut and pinned in cross section. Each preparation was loaded with the Ca^{2+} indicator Fluo-4 to reveal spontaneous activity in ICC and muscle. **Results:** We show that the human jejunum, which has a thicker LM and CM, to have a complex 3-D ICC-My network that is contiguous with ICC within the septa (ICC-SEP) that forms a clasp-like arrangement around a muscle bundle. ICC-My generated spontaneous, rhythmic, biphasic Ca^{2+} transients (6.0 ± c/min) or slow waves. Slow waves in ICC-My consisted of a rapid upstroke phase followed by a more sustained plateau phase that preceded activation of spike like activity in the LM or CM. Both phases of the slow wave propagated along a similar path through the ICC-My network but at different velocities (upstroke ~3mm/s; plateau ~300 μm/s). Activity in ICC-MY then invaded deep into the ICC-SEP, decrementing in amplitude from the myenteric region, where it activated Ca^{2+} spikes and contraction a muscle bundle. Thus, activation of ICC-SEP ensures that a whole CM bundle (or several adjacent muscle bundles) is excited during segmentation, analogous to the Purkinje system in the heart.

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Loss of Intramuscular Interstitial Cells of Cajal and Enteric Nerves in Streptozotocin-Induced Diabetic Rat Stomach

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Gastroparesis is a common condition and associated with debilitating symptoms in patients suffering from long-standing diabetes mellitus (DM). The cellular abnormality in diabetic gastroparesis has not been thoroughly studied. A decrease in the number of interstitial cells of Cajal (ICC) and nerves have been reported. However, there are different populations of ICC and nerves which serve different functions. **Aims/method:** We studied changes in the density and structure of different populations of ICC and enteric nerves in the streptozotocin (STZ)-induced DM rat stomach using c-kit and PGP 9.5 immunohistochemistry as well as electron microscopy (EM). DM was induced in Wistar rats (n=4) by a single intra-peritoneal injection of STZ (65 mg/kg) and verified by blood glucose level. Differences were compared to control rat stomach (n=4, received vehicle injection without STZ). **Results:** In DM rat stomach, the density of ICC and enteric nerves within the entire musculature were significantly reduced in both the fundus and corpus ($p < 0.05$). However, the density of ICC and nerves associated with the Auerbach's plexus (ICC-AP) was not significantly different in the corpus. The density of intramuscular ICC (ICC-IM) and nerves within the circular and longitudinal muscle layers, excluding the AP, were significantly reduced by 44.7% in the fundus ($p=0.019$) and 60.5% in the corpus ($p =0.01$). Under EM, ICC and nerves associated with AP showed either normal structure or relatively minor degenerative changes. Various degrees of degenerative changes were frequently observed in ICC-IM: swollen mitochondria, presence of lamina bodies, partial depletion of cell bodies or processes and loss of synapse-like connection with the enteric nerves. Enteric nerves also frequently displayed remarkable degenerative changes, such as swollen mitochondria. **Conclusion:** Our data demonstrated that, in the STZ-induced DM rat stomach, the damages of ICC and nerves are preferentially limited to those within in the muscle layers but not those associated with the AP, indicating that the ICC-IM and their associated nerves are the first to be damaged in DM and likely responsible for the initial motor changes. Hence, DM patients' symptoms may be related to morphological abnormalities in ICC-IM leading to impaired gastric accommodation. Delayed gastric emptying may occur later when ICC-AP are affected after a more prolonged period of hyperglycemia and further studies correlating these chronological morphological damages and changes in motor functions are warranted. Supported by CIHR and CAG.

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Postjunctional neurokinin responses persist in the absence of intramuscular interstitial cells of Cajal in the stomach

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Morphological studies have shown intimate relationships between enteric motor terminals and intramuscular ICC in several regions of

the gastrointestinal tract. Functional studies of mice lacking intramuscular ICC (ICC-IM) have suggested that ICC mediate cholinergic and nitrergic motor neurotransmission in the lower esophageal and pyloric sphincters, in the stomach and small intestine. The aim of the present study was to determine whether any post-junctional neural responses can persist in the absence of ICC-IM in the stomach.

Electrophysiological studies revealed that neural responses in the gastric antrum in response to electric field stimulation (EFS, 0.3 ms, 1-20 Hz for 1 s), consists of an IJP followed by phase advancement of the slow wave immediately after EFS. At higher stimulation frequencies (10-20 Hz) membrane potential depolarized and the duration of slow waves was enhanced for the several slow wave cycles following EFS. The fast IJP was blocked by apamin (0.2 μM) and TTX (1μM). A component of the membrane depolarization and increase in slow wave duration was insensitive to atropine but inhibited by TTX. In W/W^V mutant mice that lack ICC-IM, EFS caused a similar response to that of wildtype muscles (i.e. at 5 Hz and greater, phase advancement of the slow wave event immediately following EFS and a summation of two or more slow waves after EFS). These responses were also abolished by TTX. These data suggest that a non-cholinergic excitatory neurotransmitter is released in sufficient concentrations to excite the stomach at frequencies > 5 Hz. We explored the hypothesis that the non-cholinergic neurotransmitter could be a neurokinin in electrophysiological and isometric force experiments. In muscles of wildtype and W/W^V mice, the NK2 antagonist SR-48968 (1μM) partially inhibited EFS evoked responses observed after L-NA and atropine. SR-48968 partially inhibited the phase advancement in slow waves observed in antrum under the same conditions. The NK1 antagonist GR-82334 (1 μM), added in the continued presence of the NK2 antagonist, inhibited remaining responses to EFS in both corpus and antrum. These data suggest that higher frequencies of enteric nerve stimulation, neurokinins can be released that can directly affect smooth muscle cells or reach ICC-MY in the antrum and are not dependent on ICC-IM. Supported by NIH DK57236.

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Proliferation of the interstitial cells of Cajal is induced by serotonin through 5-HT2B receptors

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Background: Interstitial cells of Cajal (ICC) are required for normal gastrointestinal motility. Loss of ICC is associated with several human and mouse motility disorders. Other than the c-Kit-Steel factor signaling pathway, insight in the mechanisms modulating ICC survival and proliferation is lacking. **Aim:** Test the hypothesis that 5-HT, through the 5-HT2B receptor has a growth-factor-like function on the survival and proliferation of ICC. **Methods:** Expression of the 5-HT2B receptor was examined using PCR on single and sorted ICC and by RT-qPCR comparing WT versus W/W^v mouse fundus. The effect of 5-HT receptor ligands on ICC numbers were investigated in primary cell cultures. Ki67 was used as a proliferation marker. **Results:** 5-HT2B mRNA was detected in single and sorted ICC. 5-HT2B message was decreased in W/W^v fundus muscle strips compared to WT. Addition of 5-HT (1 μM) to primary cultures from mouse small intestine resulted in a 66±9 % increase ($p<0.005$) in ICC number. The effect of 5-HT was antagonized by ritanserin (5-HT2 receptor antagonist) and SB204741 (5-HT2B receptor antagonist). The 5-HT2B receptor agonist BW723C86 caused a concentration dependent increase in ICC number (50±6 % at 50nM, $p<0.04$) and an increase in ICC proliferation (32±25 % at 10nM, $p<0.02$). **Conclusion:** The 5-HT2B receptor is expressed on ICC. Activation of the 5-HT2B receptor regulates ICC numbers by increasing proliferation of ICC. These data suggest that 5-HT, through 5-HT2B receptors, plays a role in regulating the development and maintenance of ICC networks. Supported by NIH DK52766 and DK57061

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Effect of the protein and complex B vitamins deficiency on the area of the soma and nucleus of myenteric neurons of the descending colon of rats

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This research studied the effects of protein and complex B vitamins deficiency on the nucleus/soma ratio, as well as the degree of correlation between these cellular components, of myenteric neurons of the descending colon of rats. Sixteen rats (*Rattus norvegicus*) were divided in two groups, one (n = 8) fed with 22%-protein-chow (control) and the other (n = 8) with only 8%-protein-level without complex B vitamins supplementation (experimental), during 120 days. The whole-mounts of the descending colon were stained either with NADPH-diaphorase or NADH-diaphorase technique. We measured the nucleus' and soma's area of 300 neurons of each animal. The neuronal nucleus/soma ratio evidenced by NADH-diaphorase technique did not present significant difference ($p=0.6502$) between the groups. For the NADPH-diaphorase positive neurons, however, this ratio was extremely different ($p<0.001$). The number of NADPH-diaphorase positive neurons with nucleus/soma ratio lesser than 0.3 was predominant in the group control. Otherwise, the number with a ratio between 0.61-0.7 was higher in the experimental group. In both groups and techniques, we verified strong correlation between the nucleus' and soma's area ($p<0.0001$). The neurons evidenced by the NADPH-diaphorase technique presented greater average of nucleus' and soma's area than those evidenced by the NADH-diaphorase technique. The nuclei of the NADH-diaphorase positive neurons tended to be bigger than the NADPH-diaphorase positive neurons. The soma's area of the NADH-diaphorase positive neurons was not modified by the treatment. However the nucleus' area tended to decrease, even without significant changes in the nucleus/soma ratio, maintaining the positive correlation. The soma's area of the NADPH-diaphorase positive neurons of the experimental group reduced more intensely than the nucleus' area nucleus, increasing the nucleus/soma ratio, even so keeping the strong positive correlation ($r = 0.9311$). The decreasing soma's area probably reflects a lesser lowering in protein synthesis. This is possibly an adaptation process for the survival of these neurons, since they have low index of cellular turnover. Our data suggest that the NADH-diaphorase positive neurons were more resistant to reduction of the area of the soma caused by the protein malnutrition and vitamin deficiency than the NADPH-diaphorase neurons.

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Simulating virtual electrogastrograms in a normal and diseased model

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Aim: We present the first simulation study using anatomically realistic torso models and realistic dipole sources to compute virtual electrogastrograms (EGGs) using three different electrode placement configurations. EGGs representing normal slow wave activity and functional electrical uncoupling were compared for the different electrode configurations. **Background:** Despite the EGG being discovered over 80 years ago, it has not gained widespread clinical acceptance. As there is no standard electrode configuration, it is difficult to compare EGGs obtained from different research centers and to create comparative databases. There is also debate about whether EGGs are capable of detecting certain abnormal electrical events, e.g., when the corpus and antrum both pace at 3 cycles per minute (cpm). **Methods and Results:** Anatomically realistic stomach and torso models have been constructed from the visible human project and patient CT data. Temporally and spatially varying dipole sources representing gastric slow wave activity were generated from the stomach model and then used to compute potentials on the body surface via the torso model. One set of dipole sources corresponded to normal slow wave activity and another corresponded to electrical

activity with functional uncoupling between the antrum and corpus. Virtual EGGs were calculated at electrode locations on the body surface according to three different electrode placement strategies of Chen (Am J Physiol 1999), Mintchev (Gatroenterology 1993) and Koch (Oxford University Press 2004) and compared visually and quantitatively. **Discussion:** We present the first use of anatomically realistic torso models to simulate EGGs according to different electrode placement strategies. This framework allows EGGs corresponding to different electrode configurations to be compared and opens the opportunity to develop electrode placement strategies which maximize their information content.

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Gastric myoelectrical activity in acid and mixed reflux induced gastroduodenal inflammation

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Introduction: The upper gastrointestinal inflammation can induce gastroduodenal motility dysfunction. **Aim:** The aim of performed study was to investigate gastric myoelectrical activity in acid and mixed induced inflammation of the upper GI tract. **Material and Methods:** 15 male 24 hours fasted Wistar rats with free access to water were used. Three groups of rats were studied involving acid reflux (AR), mixed reflux (MR) and sham operated (SO). Acid reflux was induced by pyloric ligation which produced so-called Shay's ulcers. Mixed reflux was achieved by jejunal ligation 1cm distal to Treitz ligament. Following laparotomy similar surgical manipulations were performed without pylorus or jejunum ligation in sham-operated rats. Three electrodes were implanted: one on the gastric fundus and the second one on 2/3 distal part of the greater curvature of the stomach. The reference electrode was placed in the abdomen muscles. The slow wave recordings were performed the next day after the surgery. Captured signal was filtered with digital bandpass filter 0.01-1,2 Hz and was down-sampled at 100 Hz. Gastric and duodenal mucosa inflammation was confirmed in histopathologic assessment. **Results:** The frequency of the gastric slow wave in both AR and MR rats was higher in comparison to SO group. In SO rats it was 0,069±0,006 Hz while in the AR group it was 0,086±0,011 Hz; p<005. The value of this parameter in MR rats achieved 0,075±0,006 Hz; p>0,05.
Conclusions: The most profound gastric slow wave pathological alternations were observed in AR rats. It may suggest that an excessive acidification impairs gastric myoelectrical activity. The mixed-reflux induced gastric damage produces less emphasized changes in the gastric slow wave frequency. It seems that gastric motor activity is more disturbed in pure AR rats.

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Effect of protein and complex B vitamins deficiency on the morphoquantitative features of the myenteric plexus of the ascending colon of adult rats

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This study was performed with the purpose of studying the effects of protein desnutrition and complex B vitamins deficiency on the myenteric plexus of the ascending colon of *Rattus norvegicus*. Twenty rats were divided in two groups, one of them fed with chow having the protein level of 22% and the other fed with chow with protein level of 8% without complex B vitamins supplementation, during 120 days. The whole-mounts of the ascending colon were stained either with Giemsa or with the NADH-diaphorase technique. When observing the neurons evidenced by the NADH-diaphorase technique we noticed their predominance in the antimesocolic region of both groups. The smaller density in the lateral (intermediate) regions of the intestinal circumference may be related to the fact that the ganglia are located deep in the thick muscle layer, which would act as a barrier to the diffusion of the reagents. In the Giemsa technique, on the other hand, the segments, already dissected, are exposed to the stain for 18-24 hours, and certainly there is a better diffusion. The disnurtured rats showed a body weight 14.8% smaller than the control group and area of colon of the experimental group was 54.2% smaller while the mean neuronal density was 26.7% greater with the Giemsa technique and 27% greater with the NADH-diaphorase technique. As the decrease in area was not accompanied by an inversely proportional increase in neuronal density, it is suggested that the experimental condition led to loss of myenteric neurons. When analyzing the neuronal size, verified a significant increase in the number of small neurons and a decrease of large neurons in the experimental group compared to the control. The decrease in body weight, intestinal area and number of enteric neurons demonstrate that the reduction in the protein level in the diet impaired the supply of essential aminoacids for the synthesis of structural proteins, resulting in a smaller physical development of the animal. Likewise, the shortage of these proteins may have interfered with the repair and renewal of cytoplasmic organelles of the neurons, an event that could lead to acceleration of the processes of atrophy, aging and cell death. A deficiency of B1 vitamin leads to diminished activity of three essential RNA-producing enzymes, reinforcing the hypothesis that the reduced metabolic rate in the enteric neurons is the cause of their smaller cell size.

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Inhibition of blood vessel development in the gut leads to intestinal aganglionosis

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The enteric nervous system (ENS) arises from neural crest-derived cells that migrate along the length of the intestine and pattern into two ganglionated plexuses within the gut wall. Failure of these processes to occur normally can lead to a variety of congenital intestinal disorders, including Hirschsprung's disease. While several factors are known to regulate the migration of enteric crest-derived cells, the signals that guide their patterning into concentric submucosal and myenteric plexuses are unknown. We examined the temporal and spatial development of the ENS and blood vessels in the developing quail intestine. Endothelial cells appear in the intestine in a craniocaudal pattern and are present in the colorectum on embryonic day #4 (E4). The developing blood vessels pattern into two rings of endothelial cells within the intestinal mesenchyme at E5, 24 hours prior to the arrival of migrating neural crest-derived cells. As the crest cells arrive in the colorectum, they migrate immediately adjacent to the endothelial cells, leading to the hypothesis that the embryonic gut vasculature may serve as a substratum for the directed migration of enteric crest cells. To test this hypothesis, we treated intestinal organ cultures with SU5416, an angiogenesis inhibitor that inhibits the vascular endothelial growth factor (VEGF) receptors. SU5416 led to the absence of blood vessels in the gut and resulted in intestinal aganglionosis. These results suggest an essential role for blood vessel development in migration and patterning of the ENS. Recognizing the critical importance of the intestinal vasculature in ENS development may provide insights into the pathophysiology of neurointestinal diseases and offer potential therapeutic options for modulating ENS formation.

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Pharmacological study of nicotinic acetylcholine receptors mediating intestinal peristalsis

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Propulsive motility in the intestine is inhibited by hexamethonium-induced blockade of nicotinic acetylcholine (nACh) receptors which mediate neuro-neuronal transmission in the enteric pathways underlying peristalsis. nACh receptors are ligand-gated pentameric ion channels, but the subunit composition of the enteric nACh receptors governing peristalsis is not known. This study examined the effects of

subunit-selective and nonselective agonists and antagonists at nACh receptors on peristalsis.

Ileal segments were mounted in organ baths containing oxygenated Tyrodés solution (30 ml, 37 °C). Prewarmed Tyrodés solution was infused into the intestinal lumen (0.5 ml min^{-1}) and after leaving the segment directed into a vertical outlet tubing which ended 4 cm (= 400 Pa) above the fluid level of the organ bath. This arrangement caused a gradual rise of the intraluminal pressure to a threshold (peristaltic pressure threshold, PPT) at which an aborally moving wave of peristaltic contraction was triggered. The PPT (Pa) was used to quantify the effects of drugs which were administered directly into the organ bath. RJR 2403 fumarate (1-30 μM), a highly selective agonist at $\alpha_4ß_2$ nACh receptors, and PNU 282987 (0.1-10 μM), a highly selective agonist at α_7-nACh receptors, failed to alter peristalsis. In contrast, mecamylamine (0.01-10 μM), a selective antagonist at $\alpha_4ß_2$ nACh-receptors, led to a concentration-dependent increase in PPT and abolished propulsive motility at 10 μM. Methylcacotinine (0.1-10 μM), a selective antagonist α_7-nACh receptors, likewise inhibited peristalsis and at 10 μM caused peristalsis to cease in 4 of 6 segments. The nonselective agonist nicotine (0.01-10 μM) caused a concentration-dependent increase in PPT, with a complete inhibition of peristalsis at 10 μM in all segments tested. Pretreatment with naloxone (0.5 μM), a pan-opioid receptor antagonist, or apamin (0.5 μM), a blocker of small conductance Ca^{2+}-activated K^+-channels, prevented 3 μM nicotine from inhibiting peristalsis.

The results show that activation of $\alpha_4ß_2$ and α_7-nACh receptors fails to alter peristalsis. This observation and the finding that inhibition of $\alpha_4ß_2$ and α_7-nACh receptors blocks peristalsis suggest that $\alpha_4ß_2$ and α_7-nACh receptors are present in the enteric nervous system and contribute to peristaltic motor regulation in a pathway-selective manner. In contrast, the nonselective agonist nicotine depresses peristalsis, and the data obtained with naloxone and apamin indicate that this inhibitory effect of nicotine involves opioidergic as well as non-adrenergic non-cholinergic inhibitory neurons.

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Understanding the mechanisms of intestinal intussusception

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Intestinal intussusception is the telescoping of one segment of the bowel into an adjacent segment. It is the leading cause of intestinal obstructions in young children worldwide and can be life threatening. In the US, obstructing intussusceptions occur in approximately 1:1,000 children with an approximately 2:1 male to female bias. Identified risk factors for intussusception in children include genetics, diet, environment, and infections. The risk factors for and the mechanism of intussusception are not experimentally defined. We hypothesized that intussusception results from a segmental dysregulation of intestinal motility due to a localized inflammatory response or alteration of the enteric nervous system that is mediated by toll-like receptor 4 (TLR4) pathway signaling. Intestinal intussusception is induced in adult mice 6 hours post intraperitoneal administration of *E. coli* lipopolysaccharide (LPS). The rates of intussusception significantly differed in male (38.5%) and female (55%) mice ($p<0.005$) following a 12 mg/kg dose of LPS, indicating that intussusception is sexually biased. However, the magnitude of this bias was influenced by both LPS dose and environment. Intussusception rates varied significantly among mouse strains [BALB/c mice (55%) > CD-1 mice (12%) >C57Bl/6 mice (0%) ($p<0.005$)], indicating a genetic susceptibility to development of intussusception. TLR4 signaling was critical for intussusception; mice lacking LPS-binding protein or CD14, or with a mutant TLR4 gene did not develop intussusception. Development of an *in vivo* imaging method has been done using magnetic resonance imaging (MRI). Formation and resolution of intussusceptions and intestinal contractions were visualized in vivo using MRI. We have determined that significant intestinal motility remains following long-term exposure to isoflurane anesthesia. Therefore, MRI will be used to determine the kinetics of intussusception and localized intestinal contractility rates in live, anesthetized mice longitudinally over the 6 hours post LPS-injection. Future experiments will determine the contribution of overall intestinal motility to developing intussusception and the cellular mediators of LPS-induced intussusception.

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Effects of electrical stimulation on uterine motility to delay preterm labor

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Objective: To determine the effects of electrical pulses on inhibition of uterine contractility and delaying the delivery time in preterm model of pregnant rats. **Materials and Methods:** Eight timed-pregnant Sprague-Dawley rats were used. Uterine horns were implanted with telemetry transducer/transmitters to measure uterine EMG. Insulated electrodes were sutured onto the same bicornuate uterine horn on day 14 of gestation. An on-line computer and data acquisition system collected the transmitter signals. On day 18, 4 mg of mifepristone was injected subcutaneously in order to increase the uterine contractility.Using a stimulator, automatic and continuous electrical current was continuously and automatically injected into the uteri of 4 test animals from day 19 until the time of delivery. The electrical pulses included injecting current for a period of 10 seconds on/off, in an alternating fashion, with current having a frequency of 30Hz, 28msec duration, and 3 mA constant current. EMGauc was calculated offline for 10 individual EMG bursts in all animals (control untreated and pulsed) at 4 time-points: 2 hours after the start of recording, 7 hours after the start of recording, 1 hour prior to delivery of the control and during delivery of the control. The number of uterine electrical bursts per minute during each stage of gestation was also noted, as was the duration of the contractions. The day and time of delivery were noted. Student's t test was used to determine statistical differences. **Results:** The control unpulsed animals delivered earlier (17.87±4.76) whereas the electrical pulsed group delivered later (48.37±6.7) (Mean difference of 28 hrs between two groups, $p<0.001$). At 1 hour prior to and during delivery of the unpulsed controls, the number of bursts per minute was significantly ($p <0.001$) higher (0.73±0.09) than in the pulsed group (0.34±0.04). Similarly, the duration of contractions in the control animals (42±3.46 sec.) was significantly longer ($p<0.001$) than in the pulse treated group (26.25±3.30 sec), and the EMGauc (area under the EMG signals) in the control animals (172.5±62.91) was significantly ($p<0.03$) higher than in the pulsed group (64.25±43.63). **Conclusions:** Application of electrical pulses with specific parameter inhibits electrical activity and contractility of the pregnant uterus and thus delays preterm delivery. This electrical inhibitory method could be extremely useful to inhibit preterm labor when appropriate and may be helpful to prevent preterm labor in humans.

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Method for assessing the peristaltic impact on a magnetic capsule navigation system

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Introduction Capsule endoscopy is a novel imaging technology that relies on natural peristalsis. The free-floating nature of the capsule creates problems due to the lack of positional control [1]. Attempts have been made to develop a capsule navigation system for endoscopy, but the largest external disturbance precluding full utilization of this approach has always been the natural peristaltic force. In the present study, the impact of peristalsis on magnetic levitation-based capsule navigation systems controlled by the electric current in external electromagnets is investigated. **Aim** We aimed at assessing (a) the impact of peristalsis on a levitating intraluminal capsule; and (b) the relationship between an externally applied force and the

resulting maximal lateral displacement of the capsule within the system's response time in a custom-designed experimental setup and in advanced computer simulations using Maxwell 3D (Ansoft, Pittsburgh, PA). **Methods:** An experimental setup capable of applying known lateral forces to a levitating capsule setup was designed. At the position of maximal displacement, the system requirements to regain levitation control were determined by performing a parametric analysis of the magnetic forces as a function of electromagnets' currents, geometries, and material parameters. **Results:** In the experimental setup, a lateral force of 0.42N applied on the levitating capsule was sufficient for the system not to regain control over the capsule. In the Maxwell 3D simulations of the same scenario, the lateral magnetic force was 0.39N at the maximal displacement and was indeed less than the applied force. Generalizing the Maxwell 3D modeling for an estimated, external peristaltic force of 1.5N and a system response time of 32 msec, the maximal displacement of a 15 gram capsule was calculated to be 5 cm. A simulated system with dimensions of 50 cm OD copper electromagnets with 10000 turns, 300 A, and a 10 x 10 cm iron core, and an embedded magnet of 7 mm OD in a capsule at 15 cm vertical distance from the electromagnets was shown to sustain a lateral force of 1.5N at a lateral displacement of 5 cm. **Conclusion:** A method of assessing the system requirements of a magnetic capsule guidance system has been proposed, and a set of suggested system parameters has been provided for a plausible, real-life capsule navigation system based on magnetic levitation. **Reference:** [1] Zvi F. MD et al. *Gastrointestinal Endoscopy Clinics of North America*., vol 14, pp. 219-227, 2001. (This research was funded in part by Sandhill Scientific Inc., CO.)

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DSS-induced colitis is not aggravated by chronic stress in mice

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Studies in rats suggest that stress might act as a contributing factor exacerbating intestinal inflammation (*Autom Neurosci* 124:56, 2006). **Aims:** To evaluate if chronic psychological stress (water avoidance stress, WAS) is a valid stressor in mice and to test whether or not DSS-induced colitis is influenced by WAS. WAS influence, alone or with colitis, on colonic sensitivity was also assessed. **Methods:** Female C57Bl/6 mice were subjected to two separate protocols. In the first protocol, mice were exposed to 3% DSS for 5 days, followed, 19 days later, by WAS (1h daily for 10 days) or sham-stress. In the second protocol, mice were first subjected to WAS or sham-stress (1h daily for 10 days) and immediately thereafter received 3% DSS for 5 days. Faecal pellet output during WAS was used as a marker of stress. Clinical signs of inflammation (faecal scoring and changes in body weight) were assessed daily. After finishing the protocols mice were euthanized and colonic inflammation evaluated macroscopically. Along the experiments, colonic sensitivity was assessed by recording the responses to colorectal distension (CRD, 10-60 mmHg). Parallel groups of mice with the same treatments, were euthanized on predetermined days for histological scoring. **Results:** Exposure to 3% DSS induced a mild colitis with a peak inflammatory response between days 6 and 10 and a maximal body weight lost of about 5% at day 7. The inflammation persisted, although with lower scores, through the experimental time (up to day 34). Exposure to WAS resulted in a 2-fold increase in pellet output, maintained through the 10 days of stress. Inflammatory scores at the end of the stress period were similar in sham-stress and WAS groups. In naïve mice, repeated WAS significantly increased pellet output through the 10 days of stress. Post-stress exposure to 3% DSS resulted in a mild inflammatory response, similar to that observed in the first experiment. Throughout the study, no differences in colonic sensitivity were observed, whether animals were exposed to WAS or sham-stress in combination with DSS or normal water. **Conclusions:** This study demonstrates that repeated WAS is a valid chronic stressor in mice. However, WAS did not affect the acute phase of colitis or aggravate a chronic inflammatory state. These results suggest that stress does not affect the course of DSS-induced colitis in female C57Bl/6 mice.

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Hydrogen sulfide slows intestinal transit

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Background: Hydrogen sulfide (H2S) is a toxic gas produced in the GI tract by gut bacteria. Recently, luminal perfuision with NaHS, a salt that donates H2S in solution, was shown to stimulate jejunal muscle contractions in vitro. This effect was reversed using a 5-HT3 receptor antagonist. (Bulmer et al. DDW #748, 2005). Since increased motility can accelerate or slow transit, the effect of H2S on intestinal transit is not known. **Aim:** The aim of this study was to test the hypothesis that H2S slows intestinal transit. **Methods:** 5 dogs were equipped with duodenal and midgut fistulas. With occluding Foley catheters in the distal limb of both fistulas, the small intestine was compartmentalized into proximal half of gut (between fistulas) and distal half of gut (beyond midgut fistula). Phosphate buffer alone or with test agents (NaHS, oleate, or ondansetron) was perfused into both compartments for 90 minutes at 2 ml/min. Intestinal transit across the proximal gut was measured by the cumulative % recovery of a radioactive marker from the midgut fistula during the last 30 min of the 90-min perfusion (mean ± SE). Buffer was given in both compartments as the control (Buffer-Buffer). Buffer was given in proximal gut while 60mM oleate was given in distal gut to trigger the ileal brake response (Buffer-Oleate). To test for the effect of H2S, 1mM NaHS was given in the proximal gut while either buffer (H2S-Buffer) or oleate (H2S-Oleate) was given in the distal gut. To test the regional effect of H2S, 4 dogs were given NaHS in the distal gut (Buffer- H2S). To test role of 5HT3 receptors, 4 dogs were given a bolus of ondansetron (0.7 mg/kg) in the proximal gut with 1mM NaHS while buffer alone was given in the distal gut (Ondansetron) **Results:** Intestinal transit in proximal gut was slowed by fat in distal gut as the ileal brake response (Buffer-Buffer: 53.8± 6.0% vs. Buffer-Oleate: 16.0±3.9%) ($p<0.01$). 2. H2S in proximal gut slowed transit in proximal gut (Buffer-Buffer: 53.8± 6.0% vs. H2S-Buffer: 8.4±4.3%) ($p<0.01$) 3. H2S in distal gut did not slow transit in proximal gut (Buffer-Buffer: 53.8± 6.0% vs. Buffer-H2S: 60.7±10.0%) (n.s.). 4. H2S had no effect on ileal brake (Buffer-Oleate: 16.0±3.9% vs. H2S-Oleate:4.6±2.6%) (n.s.) 5. Ondansetron did not reverse the slowing effect of H2S in proximal gut (H2S-Buffer: 8.4±4.3% vs. Ondansetron: 3.1 ± 2.7%) (n.s.). **Conclusion:** Hydrogen sulfide slows intestinal transit independent of 5HT3 receptors. This study is supported in part by R01 DK059983.

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Development of a new intestinal smooth muscle cell culture model from rat neonates

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Cell culture obtained from isolated muscle cells has become a valuable source for studies of basic physiology properties of muscle (Lieberman, 1987). In the present study attempts were made to establish a cell cultural model of smooth muscle cells (SMC) using rat neonatal intestine. Segments (1.5 cm Length) from the duodenum, jejunum and ileum were obtained from SD rat neonates (1-4 days old). Both ends of the intestinal segments were ligated using cotton thread and exposed to 0.25% trypsin for 30, 45, 60, 75 or 90 min. Following trituration and subsequent centrifugation for 5min at 1000r.p.m., cells were suspended in DMEM-HEPES supplemented with 10% FCS, 2.5% antibiotic solution, 2.5% L-Glutamine and 0.2% Amphotericin B. Cell suspension was transferred to 25cm^2 culture flasks and incubated at 37°C. Series of viability tests were carried out using the dye exclusion assay. In separate experiments tissues were exposed to trypsin at varying durations and subsequently histological procedures were applied (Herovici, 1963). The duodenum gave rise to poorly developed cultures with limited number of cells and slow growth rate, conversely, both the jejunum and the ileum yielded highly populated SMC preparations, which gradually reached confluency (n=16). Furthermore, experiments using the quantitative method of dye exclusion assay, showed a higher population of viable cells being harvested from both jejunum and the ileum (>82%), as opposed to percentage viability obtained from the duodenum (<72%). Trypsinisation times also showed

to influence cell replication rates as shorter trypsinisation times resulted in a higher number of confluent cultures, whilst longer trypsinisation times resulted less SMC preparations reaching confluency. In relation to this, cell viability assay equally supported that a higher number of viable cells (>85%) were obtained during incubations of 30, 45 or 60 min, however, further trypsinisation (75 or 90 min) led to decrease in the number of viable cells (<75%). Histology was used in order to identify the regions from which cells were being harvested in relation to the different trypsinisation times. Following a 30 min exposure to trypsin, cells from the serosa and part of the muscularis-externae (longitudinal SMC) were extracted. 45 min trypsinisation enabled harvesting of both longitudinal and circular SMC from the muscularis-externae including neurones from the myenteric plexus. Further trypsinisation led to complete removal of the submucosal layers. The present study provided evidence that our new method of culturing intestinal SMC would allow to manipulate cultures with varying population of cells that are trypsin time dependent. Lieberman et al., 1987, Am.J.Physiol., 253:C349-C363. Herovici, 1963, Inst. Gustav Roussy, France.

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Role of 5-HT_3 receptor on visceral hypersensitivity in type 2 diabetes rat model

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Gastrointestinal symptoms which involve visceral pain are common in patients with diabetes mellitus. The pathogenesis of disordered sensory function in diabetes remains unclear. Since 5-HT_3 receptor has been suggested as the key player in visceral sensory mechanisms, we aimed to investigate the role of 5-HT_3 receptor in visceral hypersensitivity using an established animal model of type 2 diabetes mellitus. A type 2 diabetes model in adulthood Sprague-Dawley rats was developed by administrating streptozotocin (90mg/kg, i.p.) to neonatal rats. Rats were sacrificed after 8 weeks and colorectal content was examined using High performance liquid chromatography and immunohistochemistry. Visceral pain/discomfort induced by rectal distension was quantified by scoring the abdominal withdrawal reflex (AWR) and by measuring the arterial pulse rate. In diabetes rats, 5-HT contents and 5-HT immunoreactive cells were increased in the mucosal layer of colorectum. Fifty seven percent of diabetes rats showed visceral hypersensitivity to rectal distension, as indicated by higher AWR score and arterial pulse rate. A 5-HT_3 receptor antagonist granisetron (10μg/kg, s.c.) inhibited the visceral hypersensitivity of diabetes rat. Normal rats injected with 5-hydroxytryptophan (5-HTP; 10mg/kg, s.c.) showed visceral hypersensitivity as much as did diabetes rats. In 5-HTP injected normal rats, granisetron inhibited visceral hypersensitivity. These results suggest that endogenous increased 5-HT induces visceral hypersensitivity by mediating 5-HT_3 receptor in type 2 diabetes rats.

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Gut-like organ differentiated from mouse embryonic stem cells

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Recently, embryonic stem (ES) cells were shown to spontaneously give rise to a functional organ-like unit, the ''ES gut'', which undergoes rhythmic contractions and is comprised of enteric derivatives of all three embryonic germ layers: epithelial cells (endoderm), smooth muscle cells and interstitial cells of Cajal (ICCs) (mesoderm), and a small number of diffusely distributed enteric neurons (ectoderm), but no ganglia. Although the motor patterns of the ES gut are not identical to gastrointestinal (GI) peristalsis, the ES gut begins to exhibit rhythmic motor activity with periodic contractions and relaxations (phasic contraction) on about day-21 of outgrowth culture. Recent investigations have demonstrated that the ICC network within the GI tract is responsible for the generation of electrical pacemaker activity. These electrical activities control the frequency and the propagation characteristics of GI motility. Enteric neurons present within the GI tract innervate the smooth muscle and are essential for peristalsis. To differentiate abundant, extensively distributed enteric neural networks, we first challenged brain-derived neurotrophic factor (BDNF). Second, we also challenged glial cell line-derived neurotrophic factor (GDNF). By adding BDNF (10^{-8}g/ml) or a higher concentration of GDNF (10^{-6}g/ml) only during EB formation, we for the first time succeeded in *in vitro* formation of enteric neural ganglia with connecting nerve fiber tracts (ENS) from enteric neural crest-derived cells in the ES gut. The ES gut with ENS exhibited strong peristalsis-like movements. Moreover, focal stimulation of ES guts with ENS elicited propagated increases in intracellular Ca^{2+}concentration ($[Ca^{2+}]_i$) at single or multiple sites that were attenuated by nicotinic or muscarinic receptor blockade, or abolished by a neurotoxin. On the other hand, imanitib, a tyrosine kinase receptor c-KIT inhibitor, might affect ICC differentiation and spontaneous motility of ES gut. These results suggest that ENS concerted with ICCs control the spontaneous motility of ES gut.This work was supported by Grants-in-aid for Scientific Research (14657311,16650090,17300130 and 18630007 for MT and 15300134 for SN) from the Ministry of Education, Science, Sports and Culture of Japan.

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Isolation of enteric nervous system progenitors from Hirschsprung's-like gut

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Aims: Isolate and characterise Enteric Nervous System (ENS) progenitor cells (EPCs) from postnatal monoisoformic Ret51 (miRet51) mice, an established model of human Hirschsprung's disease (HSCR). 2. Determine if differentiation defects in such EPCs can be rescued by genetic manipulation. 3. Develop a protocol for isolating EPCs from human postnatal gut, including HSCR. **Methods:** Cultures of dissociated myenteric plexus from miRet51 mouse gut (ganglionic segment) were generated to form EPC-containing neurosphere-like bodies (NLBs). EPCs were selectively isolated from dissociated NLBs using a GFP tagged retroviral vector followed by flow cytometry. ''Rescue'' experiments used the same GFP retroviral transgene additionally expressing the Ret9 isoform (normally absent in miRet51). Isolated GFP+ EPCs (±Ret9) were cultured and characterised using ENS lineage and differentiation markers. In human studies myenteric plexus cultures and NLBs were established from gut resection specimens from children undergoing surgery, including for HSCR, and characterised as above. **Results:** EPCs were isolated from postnatal miRet51 mouse gut. In clonogenic cultures (15 days) EPCs differentiated into glia, neurons, and mature neuronal phenotypes. However, there was a clear defect in differentiation between miRet51 EPCs compared to their wild-type (WT) counterparts. At day 5 differentiation was minimal. By day 10, 6.1±3.4% of EPCs in miRet51 colonies expressed neuronal markers compared to 12.8±5.1% in WT ones ($p<0.01$). Glial markers were expressed by 1.5±0.68% and 7.0±2.3% of colony cells in miRet51 and WT respectively ($p=0.014$). In ''rescue'' experiments there was no statistical difference in differentiation between WT and miRet51 colonies. NLBs were generated from human tissue obtained from children aged 0.5 – 18 years and were positive for markers of glial and neuronal differentiation as well as ENS progenitors **Conclusions:** These findings suggest that defective EPC function may contribute to the pathogenesis of HSCR. In miRet51 mice, the delayed rostro-caudal migration of EPCs in the gut during embryogenesis, impaired responses to molecular cues such as GDNF, and our results support a critical requirement for differentiation in order to complete ENS formation. In therapeutic terms the results are very encouraging as they suggest that defective HSCR EPCs may be amenable to genetic rescue and, importantly, that EPCs can be isolated from postnatal human gut. These are essential first steps in establishing definitive therapeutic strategies for enteric neurodevelopmental disorders.

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The role of spinal-NMDA NR1 receptor expression in an IBS animal model

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Background: Patients with post-infectious IBS have been shown to have persistent visceral hypersensitivity. N-methyl-D-aspartic acid (NMDA) receptors play an important role in the development of hyperalgesia following inflammation. It is unclear, however, if changes in spinal-NMDA subunit receptor expression are associated with hypersensitivity following visceral inflammation. In the current study, we investigated the role of NMDA receptors in the spinal cord (L4-S1) of rats following TNBS colitis. We hypothesized that there will be altered expression of NMDA NR1 subunits following transient TNBS colitis in rats. **Methods:** Male Sprague-Dawley rats (150g–250g) were treated with either 20mg/rat trinitrobenzene sulfonic acid (TNBS, Sigma Chemical Co.) in 50% ethanol (n=16) or an equivalent volume of saline (n=5). The agents were delivered with a 24 gauge catheter inserted into the lumen of the colon. The animals were sacrificed 16 weeks following TNBS administration after resolution of the colitis. The spinal cord (L4-S1) was retrieved from three groups of rats. Group 1: Saline control group (n=3); Group 2: Recovered rats from TNBS injection without hypersensitivity (n=3); Group 3: Hypersensitive rats after TNBS injection (IBS) (n=3). The 2-dimensional polyacrylamide gel electrophorsis technique was used to investigate spinal-NMDA receptor activation following TNBS injection. **Results:** In both saline control and recovered rats, the NR1 receptor subunits expressed were $NR1_{011}$, $NR1_{001}$, $NR1_{100}$ and $NR1_{000}$. In the IBS-like rats, the NR1 subunits present were $NR1_{011}$, $NR1_{011}$, $NR1_{100}$ and $NR1_{111}$. **Conclusions:** Selective changes in the expression of the NR1 splice variants of the NMDA receptor occur following TNBS-induced colitis in the rat. These results suggest a role of NMDA receptors in the development of neuronal plasticity and resulting visceral/somatic hypersensitivity in this IBS animal model. These findings may lead to new therapeutic targets for the treatment of IBS. Supported by a Veterans Administration Merit Review Award (PI: GN Verne)

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Childhood Stress (CS) increases psychosomatic symptoms in patients with visceral and somatic hypersensitivity

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Background: Experimental and clinical evidence suggests that maternal separation, abuse or neglect during early life, make animals and humans less able to cope with stress as adults than control groups. Population-based studies, however, are conflicting. Patients with symptoms unexplained by physical or chemical abnormalities are called ''Functional''. Functional Disorders (FD) are characterized by varying degrees of visceral and somatic hypersensitivity associated with psychiatric symptoms. FD frequently coexist with a frequency that far exceeds chance. We have thus labeled those patients as affected with a Visceral and Somatic Hypersensitivity Syndrome (VSHS). **Aims:** To evaluate the effect of CS on psychosomatic symptoms in patients with VSHS. **Methods:** During their initial visit to the Disorders of Function Clinic, a total of 106 patients (87% female) over the age of 18 were dichotomized into those who experienced CS (N=58) and those who did not (N=48). Those whose evaluation met both the European and USA criteria for abuse were considered positive for CS. All patients were without concurrent organic disease that could interfere with their FD evaluation, and all signed informed consent. Published symptom-based diagnostic criteria were used to determine FD. Psychological symptoms (PS) were evaluated using the SCL-90-R, quality of life (QOL) was assessed using the QOL Inventory (QOLI) and pain severity was evaluated using a 10 cm Visual Analog Scale, which was further characterized as visceral or somatic. Demographic characteristics, PS, QOL and reported pain were compared by CS group using t-tests and Chi-square analysis. **Results:** There were no demographic differences by CS. However, every symptom subscale of the SCL-90-R as well as the Global Score Index (70.92 vs 65.83, p<.001) were significantly (p<.05) higher among those with CS. Two subscales of the QOLI (Community and Relatives) were also significantly lower (p<.05) among those with CS. There was no difference in reported pain severity or type by CS. Finally, patients with CS had a higher average number of FD than those without (5.9 vs 5.2, p<.05) as a greater proportion of them were diagnosed with 6 or more FD. **Conclusions:** Psychosomatic symptoms in patients with FD may be more intense when the patient also report experiencing CS.

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Antidepressant (AD) experience in patients (pts) with visceral and somatic hypersensitivity (VSH)

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Background: Functional Disorders (FD) are characterized by varying degrees of VSH associated with psychiatric symptoms and often appear as a syndrome (VSHS). Published evidence indicates that antidepressants of different chemical families have been effective in the management of FD and other pain syndromes. A serotonin-norepinephrine reuptake inhibitor (SNRI), Venlafaxine (VEN) has been approved for the treatment of Depression and Anxiety Disorders and has been reported of use in diabetic neuropathy. **Aims:** To evaluate in an open-label, observational pilot study, whether VEN, when added to existing treatment, decreased reported pain, and psychosomatic symptoms in patients with VSHS. **Methods:** The subjects of this study were 33 patients who were given VEN as part of their treatment regime for VSHS at our Disorders of Function Clinic. Another 24 patients were offered treatment with VEN but did not accept it, and 12 patients agreed to take it but didn't. The patients offered VEN are a subset of 200 patients that have been evaluated for FD at the clinic. Published symptom-based diagnostic criteria were used to determine FD. Psychological symptoms were evaluated using the SCL-90-R, quality of life (QOL) was assessed using the QOL Inventory (QOLI) and pain severity was evaluated using a 10 cm Visual Analog Scale, which was further characterized as visceral or somatic. All patients signed consent prior to study. Dosages of VEN ranged from 37.5 to 300 mg/day and the average length of treatment was 191 days. Improvement in pain experience and psychosomatic symptoms were evaluated using paired t-tests, assessing the change in measurements before and after VEN treatment. **Results:** Pain scores declined precipitously (−4.5 ± 2.8, p<.001), QOLI improved (+1.8 points, p<.001), the Global Symptom Index from the SCL-90-R declined (−10.7 points, p<.001) and systolic (−11mmHg, p<.001) and diastolic (−6 mmHg, p<.001) pressure declined following VEN treatment. **Conclusions:** In an open label study, VEN was well tolerated and may contribute to the relief of pain, a decline in psychosomatic symptoms and an improvement in QOL in patients with multiple FD (VSHS). However, social acceptance of therapy, hypervigilance and non-adherence to treatment are significant barriers in using AD treatment in the management of VSHS. Further research, including double-blinded trials, is needed to verify these findings.

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Correlation of immune markers, overlap symptoms and hypercoagulable measures in patients with gastroparesis: can we predict which patients will clot?

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Introduction: Many gastroparesis patients have multiple overlap syndromes as well as hypercoagulable states, some of which appear to have an autoimmune basis. (GE, 1999: GE: 2004).We have also reported that life-threatening venous thromboses has occurred in over 30% of some gastroparetic patient groups (NGM, 2004: 17:

35–43). **Patients and methods:** To investigate the hypothesis that autoimmune markers might be present in patients with overlap symptoms and/or hypercoagulable states, we retrospectively reviewed data from 21 gastroparesis patients seen in a 12 month period withthe following complete data: hypercoagulable and (Western)immunoblot profiles, as well as structured interviews to determine the presence or absence of the following overlap syndromes: Migraine Headaches, Fibromyalgia, Interstitial Cystitis, Endometriosis and Depression as well as any history of venous thromboses.Complete data was available on 21 of patients: 2 m, 19 f mean age 41 yrs , with underlying dx: 3 Diabetes Mellitus, 2 Post Surgical, 16 Idiopathic. Result were analyzed by Chi square the endpoint of thrombosis (Yes or No) Vs any of the other three criteria: overlap syndromes, serologic hypercoagulablemarkers, and/or extractable nuclear antigens (ENA) on immunoblot. **Results:** Of the 21 patients, 5 had a history of thromboses.Of the 16 with No history of thromboses: 14 had all three other criteria present. Of the 5 patients with a history of thromboses, 3 did not have all three other criteria. (p< 0.01 by Chi-square.)_(see table) **Conclusion:** In this group of patients with gastroparesis, the presence of overlap syndromes combined with postitive serologic markers for autoimmunity and hypercoagulability is associated with significantly less risk of thromboses. These data may offer a way to predict which patients are at highest risk for thromboses, especially for patients who may require IV access and/or surgical procedures.

Group	# hypercoag	# overlap	#immunoblot
Clot n=5	5	3	4
No Clot n=16 *	15	16	15
* p< 0.01			

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Antral gastric motility impairement and autonomic nervous system dysfunction in patients with pancreatic cancer

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Introduction: The aim of the study was evaluation influence of pancreatic carcinoma on gastric motility and autonomic system activity in patients before surgery. **Patients:** 11 pts with histologically confirmed pancreatic cancer without any endoscopic evidence of gastroduodenal obstruction and 30 healthy persons (58.1±8) were studied. Dyspeptic symptoms were observed in 40% of patients and 3% of the control group. **Methods:** After overnight fasting antral gastric myoelectric activity was captured with multichannel electrogastrography (Medtronics) together with simultaneous autonomic system activity measurements by heart rate variability (HRV) assessment (CNSystem) in basal conditions and after standard meal (Enrich®, Abbot Lab.). Liquid gastric emptying (LGE) was measured by means of ultrasonography (Epson). Radiolabelled solid meal (SGE) with gammakamera (mTc-colloid) was evaluated on separate day. **Results:** In patients group fasting EGG showed dysrrhythmia in 23±9% of recording time vs. 14±7% in the control group (p<0.05), which improved significantly after meal. Slow waves power increased was noted after meal (p>0.05). Control fasted antral area in patients group was 2.72±0.4 vs. 2.2±0.4 [cm^2] in the control group. Meal ingestion resulted in further increase of antral area to 7.8±0.9 vs. 6.9±1.5 [cm^2] in control group (p<0.05). Total LGE was 89±22 [min] in patients group vs. 65±7 [min] in the control group. SGE – $T_{1/2}$ was 56±23 vs. 39±9 [min] (p<0.05) respectively. **Short-term HRV recording:** In the IBS patients we noted decrease of all parameters of the spectral domain analysis of HRV in comparison with the control group (VLF – 464,1 vs. 906,3; LF – 325,1 vs. 811,6; HF – 569,5 vs. 854,6 [ms^2], p<0,05). **Conclusion:** Gastric dysrrhythmias are present in patients with pancreatic cancer both with and without gastric emptying delay. Antral distribution of meal suggests the presence of impaired proximal gastric response to meal due to diminished autonomic nervous system function and impaired myoelectric gastric activity in patients with pancreatic cancer.

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Effects of intraduodenal glucose and triglyceride on blood pressure, heart rate and splanchnic blood flow in healthy elderly subjects

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Postprandial hypotension (PPH) occurs frequently in the elderly and represents a major cause of morbidity and mortality. Our recent studies have established that the magnitude of the fall in blood pressure (BP) induced by enteral glucose is dependent on small intestinal delivery ie the fall is greater when gastric emptying is relatively more rapid. It has been suggested that, in contrast to carbohydrate, triglyceride has little, if any, effect on BP; this issue is relevant to the dietary management of PPH. The aim of this study was to determine the comparative effects of isocaloric intraduodenal (ID) glucose and triglyceride loads on BP, heart rate (HR) and superior mesenteric artery (SMA) blood flow in the elderly. Five healthy subjects (3F, 2M; age 68-77 yr) were studied on three, separate days after an overnight fast on which they received a 3kcal/min (2.7ml/min) ID infusion of either glucose (85g), triglyceride (10% Intralipid®) or saline (0.9%) over 90min (ie t=0-90min) followed by ID saline (0.9%) for a further 30min (ie t=90-120min). Systolic BP (SBP), HR (DINAMAP) and SMA blood flow (Doppler ultrasound) were measured between t=0-120min. There were comparable reductions in SBP during ID glucose (P<0.05) and triglyceride (P<0.05), but not saline (Fig a), but the response to ID triglyceride was slower (P=0.003) eg the maximum fall in SBP occurred at 103.5 ± 7.9 min during ID triglyceride and at 19.5 ± 4.7 min during ID glucose. There were comparable rises in HR (P<0.0001 for both) (Fig b) and SMA blood flow (P<0.05 for both) (Fig c) during ID glucose and triglyceride, but not during saline. We conclude that ID glucose and triglyceride both decrease BP and increase HR and SMA blood flow in healthy elderly subjects and that the response to triglyceride is slower; the latter may reflect the time taken for digestion of triglyceride to free fatty acids.

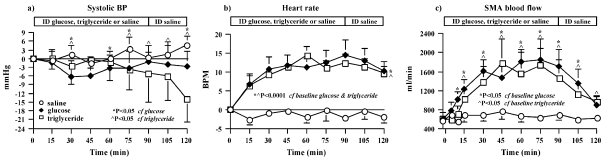

Figure 1 Effects of ID saline, glucose and triglyceride on a) SBP, b) HR and c) SMA blood flow in healthy elderly subjects. Data are mean values ± SEM.

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Gastric electrical stimulation & sacral electrical stimulation: Are two devices better than one?

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Introduction: Gastric Electrical Stimulation (GES) is available as a Humanitarian Use Device for patients with the symptoms of gastroparesis and is effective in reducing gastrointestinal symptoms. We have previously shown that patients with gastric motor disorders often have co-existing abnormalities of the genitourinary system (Gastroenterol 112: A737, 1997), which may now be treated with sacral

electrical stimulation (SES), resulting in similar improvements in genitourinary symptoms. **Patients:** We compared the results of therapy with GES and SES in 14 patients who were implanted with both devices. Patients were 12 f, 2 m, mean age of 41 years, who had documented gastroparesis as well as bladder or other pelvic floor dysfunction. All 14 patients had received their GES before the SES. **Methods:** Patients were evaluated at baseline and latest follow up (median 4 years for GES and 2 years for SES), according to previously standardized scores of GI (GI: 0-4, TSS max 20) and GU (GU: 0-3, UTSS, max 12) function. Results were compared by paired t-tests and reported as mean ± SE. **Results:** All 14 patients improved both GI and GU symptoms and the improvement in all parameters as nausea, vomiting, anorexia, bloating, abdominal pain and gastric total symptom score (TSS), leakage, urgency, voiding difficulty, number of pads used and urinary total symptom score (UTSS) were statistically significant (see table below). **Conclusions:** Combinations of GES & SES appear to be safe and effective for patients with both gastroparesis and bladder dysfunction and the existence of a stimulator for one disorder does not preclude another stimulator.

	V	N	A	B	ABD. PAIN	TSS
Before	2.8±.6	3.9±.1	3.5±.1.1	3.6±.0	3.8±.1	16.0±.1.1
After	.63±.2	1.0±.1	1.6±.1	1.9±.4	1.3±.3	6.6±.9
P Value	<.001	<.001	<.001	<.002	<.001	<.001
	LEAK-AGE	URGENCY	VOIDING DIFF.	# PADS	UTSS	
Before	1.33±.49	2.0±.51	2.42±.43	0.83±.48	6.5±.1.28	
After	0	.67±.42	.5±.31	0	1.4±.75	
P Value	<.001	.007	0.002	.007	<.001	

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Stress associated changes in the epithelial function and luminal microbiota in the human jejunum

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Several evidences support the relevance of intestinal flora and innate immunity in the development of mucosal inflammatory disorders in the gut whereas acute stress is known to reactivate these disorders. However, the effect of stress on these factors has not been fully elucidated. **Methods:** In 25 healthy volunteers, a 20-cm jejunal-segment perfusion was performed. After 30 min of equilibration, intestinal effluents were collected every 15 min, for 30 min under basal conditions and for 1h after 15 min of cold pain stress. Epithelial responses including net water flux and macromolecular permeability to albumin were determined along with autonomic (blood pressure and heart rate), as well as psychological responses (analogic scale) to stress throughout the study. Dominant bacterial populations in intestinal effluents were detected by PCR-DGGE fingerprinting at baseline (A) and 45 min after stress (B). Obtained profiles were compared and similarity indices (SI) calculated from densitometric curves using the Pearson product moment correlation coefficient. Human defensins (HNP 1-3) release was determined under basal conditions and after acute stress. **Results:** Cold pain stress markedly increased heart rate, blood pressure and psychological response. A significant increase in net water flux was observed after cold pain stress. Total bacterial DGGE profiles showed a low interindividual SI at baseline, but this SI was significantly ehhanced after stress. A specific band that was nearly undetectable in basal samples appeared with high intensity after acute stress. The intra-individual composition of the microbiota varied notably after cold stress and was associated with an enhancement in the release of defensins to the intestinal lumen. **Conclusions:** Acute stress affects jejunal mucosal microenvironment by inducing changes in the small bowel microflora that are associated with activation of epithelial and immunological responses. These effects might contribute to our understanding of gastrointestinal disorders caused or aggravatted by life stress.

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The link between meal related symptoms and cytokine release in patients with functional dyspepsia

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Background: Immune responses are believed to play a critical role for the manifestation of functional gastrointestinal disorders. In recent studies we have demonstrated (AJG 2004;99(4)703-10) differences in systemic cytokine levels after a meal. The current study aimed to assess the link between baseline and lipopolysacharide (LPS) stimulated cytokine levels and the symptom pattern in patients with functional dyspepsia. **Methods:** 22 (H. pylori-negative) patients with functional dyspepsia according to ROME II (8 males, aged 21- 72 mean 46.3) were recruited after a standardized diagnostic work-up. Symptom pattern and severity of symptoms were assessed utilizing the BDQ (Bowel Disease Questionnaire). Peripheral blood mononuclear cells (PBMC) from 40 cc whole blood were freshly isolated by density gradient centrifugation and cultured for 24 hours in RPMI 1640 supplemented with 10% FCS. Basal and E. coli LPS (1-100ng/ml) stimulated pro-inflammatory cytokine production (TNF-α, IL-1β, IL-6) of PBMC was measured by enzyme-linked immunosorbent assay (ELISA). **Results:** Overall, intensity of abdominal pain or discomfort were significantly associated with baseline TNF-α ($r=0.70$, $p<0.001$), IL-1β ($r=0.54$, $p<0.001$) and IL-6 ($r=0.51$, $p<0.002$). The release of TNF-α, IL-1β and IL-6 were significantly higher in patients reporting early satiety and meal related pain (TNF-α $p<0.001$; IL-1β $p<0.01$; IL-6 $p<0.05$) compared to patients without these symptoms. Cytokine release was not linked to nausea or vomiting. **Summary and Conclusion:** In-vitro-release of cytokines from PBMC is linked to severity of symptoms and in particular associated with meal related complaints. Based upon this data we conclude that immune responses that are mediated by cytokine release are linked to the symptom pattern in patients with functional GI disorders.

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Adrenergic and cholinergic modulation of Immunoglobulin A (IgA) secretion in mucosal explants from porcine colon

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Secretory IgA is a critical factor in the first-line defense against pathogenic microorganisms at mucosal surfaces in the intestinal tract. At gut immune effector sites, crypt epithelial cells adjacent to IgA^+ plasma cells in the lamina propria transport IgA into the lumen by means of the polymeric Ig receptor. Luminal IgA contains secretory component (SC), a remnant of the Ig receptor. We tested the hypothesis that intestinal IgA secretion is modulated by enteric neurotransmitters. Colonic mucosal explants were obtained from barbiturate-euthanized pigs (15 - 18 kg b. wt.). In immunohistochemical experiments, 20 micron cryostat sections of fixed colonic explants were labeled with antisera directed against the vesicular ACh transporter and dopamine *beta*-hydroxylase (used to visualize cholinergic and adrenergic nerve fibers, respectively) in combination with anti-IgA and anti-SC antisera. These experiments demonstrated that both cholinergic and adrenergic nerve fibers were situated in close proximity to IgA-positive B cells and SC-positive epithelial cells. IgA immunoreactivity in the luminal bathing medium was measured by ELISA before and after contraluminal drug addition in mucosal explants mounted in Ussing chambers. Within 30 min, the cholinergic agonist carbachol (10 μM) nearly doubled luminal IgA secretion; its effects were prevented by the muscarinic antagonist atropine. Norepinephrine (10 μM) increased IgA secretion by 3-fold; its effects were prevented by the *alpha*-adrenoceptor blocker phentolamine. In Western blots, IgA-immunoreactive material recovered from the luminal medium resolved at the same molecular weight as SC immunoreactivity, confirming its identity as

secretory IgA. Furthermore, the amount of free SC increased after carbachol or norepinephrine treatment, suggesting that these neurochemicals act upon polymeric Ig receptor-expressing epithelial cells to promote mucosally-directed IgA transport. In conclusion, adrenergic and cholinergic nerves projecting to the gut lamina propria may act to phasically modulate IgA release and likely reduce the incidence of mucosal infections. Supported by NIH/NIDA grant R01 DA-10200.

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Anatomical evidence for nerve-immunocyte interactions in peyer's patches (PP) of the porcine jejunum

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Peyer's patches, a key component of the gut-associated lymphoid tissue, serve as the primary inductive site for intestinal immunity. In the porcine jejunum, they appear as aggregates of lymphoid follicles covered by a modified epithelium. Specialized cells within the follicle-associated epithelium capture antigens from the intestinal lumen and transport them in the subepithelial dome, where they are processed by antigen-presenting cells. Several previous reports indicate that PPs are densely innervated. In the present study, we addressed the hypothesis that the morphological features of PP innervation are consistent with an immunomodulatory role for the enteric nervous system. Jejunal Peyer's patches were obtained from barbiturate-euthanized pigs (15 - 18 kg b. wt.) and fixed in modified Zamboni's solution. PP innervation was examined using antisera directed towards the pan-neuronal marker PGP9.5 and the neuropeptides substance P (SP), vasoactive intestinal peptide (VIP), and calcitonin gene-related peptide (CGRP). Subsets of PP immunocytes were visualized with antisera against CD3, IgA, IgM, and MHC class II. Laser scanning confocal microscopy was used to collect images through large tissue volumes in thick transverse sections (> 100 microns) and in whole-mount preparations (> 200 microns), yielding a three-dimensional perspective of the neuronal network superimposed on PP follicles. This three-dimensional view suggests that the enteric innervation of PP may provide means for coordination of immune activity at multiple subepithelial domes. In double- and triple-labeling experiments, SP- and CGRP-immunoreactivities often colocalized within ganglia and nerve fibers, but generally there was no overlap of VIP-immunoreactivity with SP or CGRP staining. In villi adjacent to PPs, SP- and VIP-immunoreactive nerve fibers were found in close apposition to IgA-positive B cells and CD3-positive T cells. Intimate associations were also observed between peptidergic nerve fibers and MHC class II-positive immunocytes in PP domes, consistent with the idea that neuronally released neuropeptides can influence the function of antigen-presenting cells. In summary, these results suggest that nerve fibers in PP may participate in neuroimmune cross-talk within individual antigen-sampling sites and integrate information across multiple antigen-sampling sites. Support: R01 DA-10200 (DRB) and K01 DA017236 (LV).

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Guinea pig esophageal mast cells: Unique characteristics of tissue distribution, subtypes and antigen-induced activation

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Mast cells (MCs) are divided into two subsets: mucosal MCs (seen in intestinal mucosa with low histamine content and high T cell dependence) and connective tissue MCs (seen in the skin with high histamine content and little T cell dependence). Whether esophageal MCs share features of mucosal or connective tissue MCs is unknown. **Aim:** to characterize esophageal MCs with respect to their distribution, subtypes (tryptase and/or chymase) and the effect of antigen challenge on MC phenotype and histamine release compared to mucosal (intestine) and connective tissue (skin) MCs. **Methods:** Guinea pigs (100-300 g) were actively sensitized by 3 i.p. injections of ovalbumin (OVA, 10mg/kg). 21 days after the last injection, the esophagus, ileum and skin were removed and prepared for immunofluorescent-staining of mast cell tryptase and chymase, before and after challenge by incubation with OVA. Histamine release was measured in the esophagus, stomach, intestine, and colon following antigen-challenge and expressed as a percent of total histamine content in the tissue (released by acid incubation). **Results:** (1)Similar to the skin, where more than 98% MCs are located in the dermis, more than 98% esophageal MCs are in the lamina propria. In contrast, ileal MCs are found both in mucosa (53%) and sub-mucosa (47%). (2) Three immunophenotypes of mast cells (MCs) are found: tryptase positive only (MCT), chymase positive only (MCC) and positive for both (MCTC). In esophagus, 62% were MCTC, 17% MCC and 21% MCT which is a similar distribution to that of the intestine (68% were MCTC with 9% MCC and 23% MCT, $p>0.05$) but different to the skin (92% were MCTC with 1% MCC and 7% MCT, $p<0.001$). (3) OVA challenge causes MC degranulation (combined numbers of MC subtypes) in the esophagus (44±12 vs. 12±4/mm2 cross section, n=6, $P<0.05$) and skin (170±37 vs. 98±24/mm2, n=6, $P<0.05$). No significant degranulation was seen in the ileal mucosa or submucosa. (4) OVA challenge released 19% of total tissue histamine content from the esophagus, compared to 3.3% from stomach, 4.8% from intestine, and 1.6% from colon. **Conclusion:** MCs in the GP esophagus are not typical of either mucosal or connective tissue MCs, having unique features in distribution, immunophenotypes (resembling mucosal MCs), and degranulation response to OVA challenge with release of significant amounts of histamine and proteases into the tissue (resembling connective tissue MCs). These characteristics may determine their function in normal and disease states.

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Gastric contents correlate to hunger and satiety sensations: Effects of ghrelin and glucagon-like peptide-1

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Background: Ghrelin stimulates gastric emptying, while glucagons-like peptide-1 (GLP-1) slows gastric emptying rate. The objective was to evaluate how gastric contents correlate to hunger and satiety sensations. The study was conducted in a randomized double-blind crossover fashion. **Methods:** Normal human volunteers were studied with saline, ghrelin (10 pmol kg^{-1} min^{-1}) or GLP-1 (0.7 pmol kg^{-1} min^{-1}) infusion for 180 min after intake of a radioactively labelled omelette (310 kcal). Gastric emptying parameters and post-prandial plasma levels of ghrelin and GLP-1 were measured. Hunger and satiety scores were studied by means of validated visual analogue scales (0-100) and correlated to gastric contents. **Results:** Normal gastric emptying during saline infusion was positively correlated to hunger (r^2 = 0.96, p = 0.003) and negatively correlated to satiety (r^2 = −0.92, p = 0.009). The emptying rate was fast and complete with ghrelin (T50 49.4 ±3.9 min) compared to saline (T50 75.6 ±4.9 min) ($p<0.001$). Hunger and satiety ratings were positively (r^2 = 0.86, p = 0.024) and negatively (r2 = −0.86, p = 0.024) correlated to gastric emptying, respectively. With GLP-1 only partial emptying to about 25% was achieved (T50 extrapolated 242 ± 30.9 min). Even though, a positive correlation to hunger was found (r^2 = 0.91, p = 0.012), while correlation to satiety was negative (r^2 = −0.93, p = 0.007). **Conclusion:** A close relationship prevails between gastric contents and sensations of hunger and satiety. This finding seems to be valid throughout the gastric emptying process as validated for hunger ratings between VAS 30-75 and satiety ratings between VAS 20-70. However, at extreme VAS ratings curves may deviate.

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Effects of low-fat foods upon gastric emptying rate and cholecystokinin secretion in man: Influence of the application form of the fat

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Digestion of nutrients in the gastrointestinal (GI) tract is tightly controlled and GI motility plays a critical role in it. The various segments of the GI tract coordinate in a complex yet precise way to control digestion, absorption and food intake. A negative feedback loop induced by digestion products along the entire small intestine controls gastric emptying (GE). Fatty acids (FA) released in the duodenum trigger secretion of gut hormones as cholecystokinin (CCK), which in turn inhibits GE. These small intestinal effects on hormone secretion are important for food intake regulation. However, the release of lipids from the stomach and the relationship of GE of the aqueous and lipid phase of a meal on satiety parameters has only been partly studied. A few studies suggest phase separation of lipids in the stomach when given in large amounts. Main objective of this pilot study was to determine whether small amounts of phase separated lipid in the stomach could influence satiety parameters differently to lipid emulsified in a drink. Six healthy young males were intubated nasogastrically on 4 different occasions (duplicate measures). In the *ON TOP* treatment subjects received orally a lipid-free meal replacement drink (325ml, 160 kcal) followed by intragastric infusion of 5g labelled canola oil (U-[^{13}C]-oleic acid), to mimic phase separation in the stomach. In the *EMULSION* treatment the drink contained 5g labelled canola oil as a fine emulsion; saline was infused as a control. Postprandial responses in dependence of *ON TOP* or *EMULSION* treatment upon GE (paracetamol absorption test), lipid absorption, CCK secretion were examined. Initial GE of the aqueous phase was equal between both treatments indicated by similar paracetamol (P) absorption lag times. However, GE slowed down in *EMULSION* indicated by lower P fractions absorbed after 30 min, although not significant different in this pilot study. A trend for slower GE was also indicated by a prolonged mean residence time of paracetamol in response to *EMULSION* delivery (p=0.10). Postprandial CCK-8 levels increased five-fold up to 1.25 ± 0.21 pmol⋆l^{-1} and were not significant different between the treatments. However, CCK-8 plasma levels tended to be higher in the in the ON TOP treatment between 1-4hrs postprandially. The first results of this pilot study suggest that minor amounts of phase separated lipids are released slower from the stomach and these amounts are also reflected by CCK levels.

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Lack of interaction between peripheral injection of CCK and obestatin in the regulation of gastric satiety signaling in rodents

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Obestatin is a new peptide for which anorexigenic and delayed gastric emptying effects were recently reported upon intraperitoneal (ip) or central injection in mice. We aimed to investigate the effects of peripheral injections of obestatin alone or in combination with cholecystokinin (CCK) on food intake, gastric motility and gastric vagal afferent activity in rodents. Changes in cumulative food intake was monitored after ip injection of obestatin (30, 100 and 300 μg/kg, (12–120 nmol/kg)) alone or with CCK (1 μg/kg, 0.3 nmol/kg) in fasted rats, and in fasted lean and high fat diet obese mice. Gastric emptying (GE) of a non caloric meal was measured in fasted rats 20 min after ip obstatin (300 μg/kg, ip) alone or with CCK (1 μg/kg (0.9nmol/kg)). Last, intragastric pressure (IGP) in urethane anesthetized rats and gastric vagal afferent activity in an in vitro rat stomach-vagus preparation were recorded in response to obsetatin (300 μg/kg, intravenous (iv) and 30 μg, intra arterial (ia) respectively) and and CCK (0.3 μg/kg, iv and 10ng, ia). Obestatin (30-100 μg/kg, ip) did not influence cumulative food intake for the 2 h post injection in rats (−14.1% for obestatin 300 μg/kg vs vehicle group; p<0.05) nor in lean (−19.5%; p>0.05) and obese mice (−1.5%; p>0.05). In addition, neither GE (56.4 ± 4.2% compared with 50.3 ± 4.4% in the vehicle group; p>0.05) nor IGP were modified by ip obestatin. By contrast, CCK decreased significantly cumulative food intake vs vehicle groups within the first 30 min by 36.6% in rats and 52.2% in lean mice. CCK also decreased significantly GE by 31.0 ± 6.4%;compared to the vehicle (p<0.05) and IGP with a peak response at 120s (11.0 ± 4.3% vs. baseline; p<0.05). In the in vitro rat gastric preparations, 20 afferent units had a non significant changes in activity after obestatin and responded to CCK (10 ng) by a 182% increase (p<0.05). Obestatin did not influence the CCK responses on food intake (−47.1 % at 2h; p>0.05 vs CCK alone), GE (26.7 ± 5.0%; p>0.05 vs CCK alone), and there was no change in IGP was elicited by obestatin injected 30 min after CCK administration. These data do not support a role of obestatin in rodent regulation of satiety signaling in rodents as shown by the lack of influences on hyperphagic response to fast, gastric motor function and vagal afferent activity or CCK actions.

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The effects of exogenous cholecystokinin-8 on antropyloroduodenal motility, appetite and energy intake are not diminished following exposure to a high-fat diet for 3 weeks in healthy males

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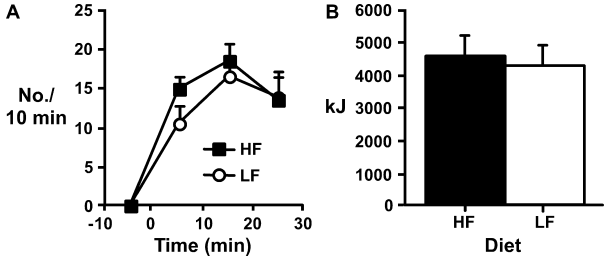
Introduction: There is evidence, albeit inconsistent, from rodent studies that the gastrointestinal effects of both fat and cholecystokinin (CCK) are attenuated by a high-fat diet. For example, suppression of gastric emptying and food intake by exogenous CCK-8 was diminished following a 34 or 54% fat diet when compared with a 5% fat diet (1). In humans, following a high-fat diet gastric emptying of fat is accelerated (2); moreover, the pyloric motor response to intraduodenal lipid is decreased following a 40% fat diet when compared with a 10% fat diet, despite similar increases in plasma CCK concentrations (3), suggesting that the sensitivity to CCK is reduced by a high-fat diet. It should, however, be noted that previous studies in humans have compared diets that were not isocaloric. **Hypothesis:** The effects of exogenous CCK-8 on antropyloroduodenal motility, appetite and energy intake will be attenuated by a high-fat diet. **Protocol:** 10 healthy lean males (age: 22.4±1 yr, BMI: 22.1±0.8 kg/m^2) consumed isocaloric diets (~15,400 kJ per day) containing either 44% (HF) or 10% (LF) fat for 21 days in single-blind, randomised fashion. The protein content of the diets was matched. Immediately following each diet, the effects of an intravenous infusion of CCK-8 (2 ng/kg/min for 45 min) on antropyloroduodenal motility and appetite perceptions (hunger and fullness) were assessed. At t=30 min during the infusion, subjects were offered a buffet-style meal, from which energy intake (kJ) was evaluated. **Results:** Body weight was not affected by the diets (HF: 69.5±22 kg, LF: 69.3±22 kg). CCK-8 decreased the number and amplitude of antral and duodenal pressure waves, and stimulated basal pyloric pressure and isolated pyloric pressure waves (*Fig A*) (P < 0.05 for all), with no difference in the response between the two diets. Perceptions of appetite and energy intake (*Fig B*) also did not differ. **Conclusions:** The effects of CCK-8 on antropyloroduodenal motility and energy intake were not affected by exposure to a high-fat for a period of three weeks in healthy lean men, indicating that sen-



sitivity to exogenous CCK-8 is not changed following a short period on a high-fat diet. **References:** (1) Covasa and Ritter, Am J Physiol 278: R166-R170, 2000. (2) Cunningham *et al*, Gut 32: 483-486, 1991. (3) Boyd *et al*, Am J Physiol 285: G188-G196, 2003.

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The effects of the fatty acid, lauric acid, on antropyloroduodenal (APD) motility and energy intake are dependent on load, but not concentration

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Introduction: We have demonstrated that intraduodenal (ID) infusion of lauric acid (C12) inhibits antral and duodenal pressures, stimulates pyloric motility, modulates gut hormone release and reduces energy intake (1,2). It is, however, unclear whether the load, or the concentration, of C12 mediates these effects. Animal studies suggest that the effects of fatty acids on gastric emptying are concentration-dependent (3), while the effect of C12 on energy intake is load-dependent (4). Our recent studies suggest that, in humans, the suppression of energy intake by C12 may be concentration-dependent (1,2). At a constant load (0.4 kcal/min), ID infusion of C12 at 106 mM suppressed energy intake much more than infusion at 56 mM, although 106 mM is supraphysiological and induced nausea. **Hypothesis:** The modulation of APD pressure waves (PWs) and the suppression of energy intake by C12 is both load- and concentration-dependent in humans. **Protocol:** 24 males (12 in each study) (age 18-36 yrs, BMI 19–25 kg/m^2) were studied on three occasions in double-blind, randomized fashion. APD PWs were assessed during ID C12 infusion at *increasing loads* (0.2, 0.3 or 0.4 kcal/min, all 56 mM) for 90 min (study 1) or *increasing concentrations* (40, 56 or 72 mM, all 0.4 kcal/min) for 60 min (study 2). Nausea was evaluated throughout. Energy intake at a buffet-meal was quantified following the infusions. **Results:** C12 0.3 and 0.4 reduced the number of antral and duodenal PWs, and stimulated pyloric PWs compared with C12 0.2 (P<0.01). C12 0.4 decreased duodenal PWs compared with C12 0.3 (P<0.05) (**Figure**). Energy intake was suppressed with C12 0.4 compared with C12 0.3 and 0.2 (P<0.01). There were no effects of concentration on APD motility or energy intake. Nausea was not experienced during any of the infusions. **Conclusion:** In contrast to our hypothesis and animal data, the effects of intraduodenal C12 on APD motility and energy intake are load-dependent, and concentration-independent, at the concentrations evaluated. **References:** (1) Feltrin *et al*, AJP 2004,287:R524-33 (2) Little *et al*, AJP 2005;289: R1090-98 (3) Lin *et al*, AJP 1990;259:G1031-36 (4) Meyer *et al*, AJP 1998;275:R1293-307.

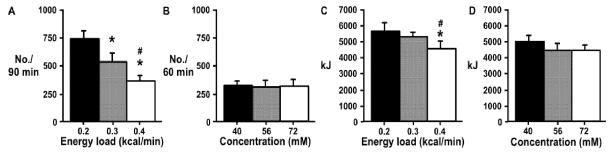

Figure 1 No. of duodenal PWs (A,B) and energy intake (C,D) during ID C12 at increasing loads (0.2, 0.3, 0.4, kcal/min) or concentrations (40, 56, 72mM). Data are means±SEM. *vs. C12 0.2, #vs. C12 0.3, P<0.01.

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Gastric emptying is not altered by chronic vagal stimulation

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Chronic vagal stimulation is commonly used to treat refractory epilepsy in humans (Wheless and Baumgartner 2004). Recently, we have shown that this method reduces food intake in pigs (Biraben et al, 2005). This diminished food intake, a potential cure for morbid obesity, might originate from an alteration in gastric function since reduced emptying is associated with early satiety in humans. Alternatively, the establishment of new neural network at the brain stem level and above might be also involved. The aim of this study is to evaluate the potential alterations in gastric emptying induced by chronic vagal stimulation in conscious pigs.

Eight pigs (40 ± 3.4 kg) were fitted with two sets of sleeve electrodes (Cyberonics) on the dorsal and ventral thoracic vagal trunks immediately before their entrance in the diaphragm. All animals were chronically stimulated for 30 sec every 300 sec using a pulse train of 0,5 to 5 mA, pulse duration from 20 to 100 ms and frequency 10 to 30 Hz. These stimulator parameters have been shown to reduce food intake after 4 weeks of continuous stimulation. Gastric emptying of liquids and solids were evaluated before and 6 weeks after the onset of the stimulation. Emptying of liquids was measured after the ingestion of 500 ml-10% dextrose labeled with 20 MBq 99mTc-DTPA. Emptying of solids was evaluated after the ingestion of 350g porridge labeled with 20 MBq 99mTc-Sulfur colloid. Nuclear images of the stomach were acquired for 40 min (liquids) and 120 min (solids) using a scintigraphy camera (Elscint) with its head located lateral to the animal resting in a sling frame. Half emptying time were derived from emptying curves fitted to a power exponential function. Differences before and after stimulation were determined by ANOVA procedure with p<0.05 considered as significant.

Gastric half emptying time of liquids was not altered by vagal stimulation (25 ± 1.0 min vs 28 ± 0.9 min, p>0.05 after vs before stimulation respectively). Similarly, half emptying time of solids was identical before and after stimulation (63 ± 0.9 min vs 61 ± 0.8 min p>0.05 after vs before stimulation respectively). Irrespective of the meal, the curve form factor (ß) remained unaffected by stimulation.

We conclude that the reduced food intake observed after chronic vagal stimulation is not related to an altered gastric emptying. On the contrary, it is likely that modifications in brain activation already observed during chronic vagal stimulation are primarily responsible for the changes in ingestive behavior.

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Peptide YY release in anorectic patients after liquid meal

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Peptide YY (PYY) is released postprandially and is dependent on the caloric content of the meal. Fasting and postprandial levels of human PYY were recently found to be lower in obesity. To clarify whether alterations of PYY play a role in the etiology of anorexia nervosa, PYY levels were investigated in anorectic patients under basal conditions and in response to liquid meal. **Methods:** We investigated PYY plasma levels in 16 female anorectic (24.6±1.2 years; restrictive type: BMI 15.2±0.3 kg/m2) and 7 lean subjects (28.9±0.9 years; BMI 21.3±0.6 kg/m2) before and after ingestion of 250 ml standard liquid meal (250 kcal: 9.4 g protein, 34.4g carbohydrates, and 8.3 g fat). PYY levels were analyzed at 0, 20, and 60 min using PYY ELISA (Diagnostic Systems Laboratories Inc., Texas, USA). Values are given as mean±SEM. **Results:** Basal PYY levels in anorectic patients were higher (89.0±14.4 pg/mL), but not significantly different from lean subjects (64.1±12.1 pg/mL). Postprandial PYY levels in healthy volunteers increased significantly after 20 and 60 min (80.4±12.7 pg/mL and 96.0±19.9 pg/mL, respectively; P=0.04). In anorectic women PYY concentrations were increased at 20 min (137.9±19.5 pg/mL; P=0.001) and at 60 min (151.3±19.2 pg/mL; P=0.001). There was no significant difference in PYY increase or in incremental integrated PYY release between both groups. **Conclusions:** We conclude that in our study basal and postprandial PYY levels in normal weight women are not different from anorectic patients. Therefore we could not confirm the recently published blunted postprandial PYY response in anorexia, a finding that merits further study.

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Prokinetic therapy is associated with a significant reduction in aspiration pneumonia in severely developmentally disabled patients on enteral nutrition

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Introduction: Patients with severe developmental disabilities (DD) such as mental retardation, cerebral palsy, and refractory epilepsy require intensive support because of their medical complexity and high demands in their daily care. Many of these patients are on enteral nutrition through either gastrostomy or jejunostomy tubes, but despite enteral feeds, 24 hour anti-reflux positioning, and good seizure control, aspiration pneumonia is common. **Patients:** To evaluate the clinical benefit of prokinetic therapy in tube fed patients with severe DD, a retrospective study was performed at a large state-run residential facility devoted to the care of DD patients. From a general pool of 325, we selected 22 patients with severe developmental disabilities who were on tube feedings and had complete data before and after cisapride therapy during 12/90 to 10/98. All patients had been placed on tube feedings using a protocol to determine the need for enteral feedings. **Methods:** Patients were followed for mean of 21.3 months before and 38.4 months after cisapride therapy. An additional standardized protocol was used to diagnose and treat aspiration pneumonia in all patients Results: The numbers of hospitalizations from aspiration pneumonia per patient month before and after cisapride administration were 0.24 (mean) +/− 0.09 (SE), (range 0.05-1.50) and 0.05 +/− 0.01 (0.0-0.24), p<0.05 with the relative risk reduction being 4.8. The number of days of aspiration pneumonia hospitalization before and after cisapride administration were 2.8 +/−1.17 (0.0-20.25) and 0.5 +/− 0.13 (0.0-2.04), p=0.06. There were no adverse events noted from the therapy; particularly no cardiac arrhythmias or significant Q-T prolongation was noted. **Conclusion:** Appropriately monitored prokinetic therapy may prove to be beneficial in this highly selected group of patients who are at a high risk for developing aspiration pneumonia. Not only are the numbers of aspiration pneumonia episodes reduced, but the number of days of hospitalizations during each episode is also reduced. Prospective studies in certain high-risk groups seem warranted.

BEFORE CISAPRIDE	MEAN	STANDARD DEVIATION	DURING CIS (MN)	DURING CIS (SD)	P. VALUE	RELATIVE RISK
HOSPITAL ADMISSIONS/YEAR	2.75	(1.05)	0.61	(0.15)	<0.05	4.8
HOSPITAL DAYS/YEAR	32.3	(14.1)	6.4	(1.6)	<.0.05	——

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Load-related effects of duodenal lipid on antropyloroduodenal (APD) motility, cholecystokinin (CCK) and peptide YY (PYY) release, appetite and energy intake in healthy men

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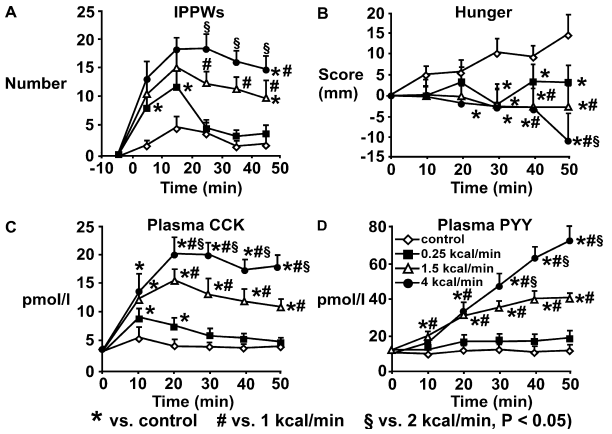
Background: We have recently reported that both load and duration of intraduodenal (ID) lipid influence APD motility as well as CCK and PYY release (Pilichiewicz et al. AJP 2006;290:R668-77). **Aims:** To determine (i) whether ID lipid loads well below the mean rate of gastric emptying affect APD motility, CCK and PYY and (ii) the effects of ID lipid load on appetite and energy intake. **Methods:** 16 healthy men (age: 32±4 yrs; BMI: 23.6±2 kg/m^2) were studied on four occasions in double-blind, randomized fashion. APD motility, plasma CCK and PYY, and appetite perceptions were measured during ID lipid (Intralipid®) infusions at: (i) 0.25 (''IL0.25''), (ii) 1.5 (''IL1.5'') and (iii) 4 (''IL4'') kcal/min or, iv) saline control (''C''), each for 50 min. Immediately after the infusions energy intake at a buffet meal was quantified. **Results:** Stimulation of isolated pyloric pressure waves (IPPWs) (*Fig A*), suppression of antral PWs and stimulation of CCK and PYY were load-dependent (P<0.05 for all) (*Fig's C, D*), although IL0.25 had no effect on PYY. There was a load-dependent suppression of hunger (*Fig B*) and desire to eat (P<0.05 for both), while only the highest dose of lipid (IL4) reduced energy intake ([kJ], C: 5384±256, IL0.25: 5367±186, IL1.5: 5161±295, IL4: 4753±273; P<0.05 vs C and IL0.25). **Conclusion:** In healthy subjects (i) ID lipid loads as low as 0.25 kcal/min modulate APD motility and CCK release, and (ii) the suppression of perceptions of appetite, but not energy intake, by ID lipid, is load-dependent, at the loads evaluated.



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Effects of variations of duodenal glucose loads on glycemia, antropyloroduodenal (APD) motility, appetite and energy intake in healthy men

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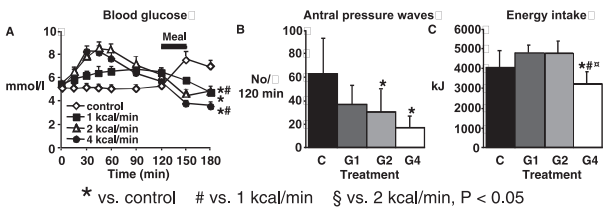
Background: As a result of small intestinal feedback glucose empties from the stomach at a rate of ~1-3 kcal/min. The rate of small intestinal glucose entry is a major determinant of the effects of a meal on gly-cemia and appetite. **Aims:** To determine the effects of different intraduodenal (ID) glucose loads on glyce-mia, APD motility, appetite and energy intake. **Methods:** 7 healthy men (29?3 yrs; BMI: 24?3 kg/m^2) were studied on four separate occasions in double-blind, randomized fashion. APD motility, blood glucose and appetite perceptions were measured during 120 min ID glucose infusions at (i) 1 (''G1''), (ii) 2 (''G2''), and (iii) 4 (''G4'') kcal/min, or (iv) saline control (''C''). The concentration of all solutions was 1390 mosmol. Immediately after the infusions energy intake at a buffet meal was quantified. **Results:** There was a rise in blood glucose *(Fig A)* during all glucose infusions (P<0.05 vs. C), with the effect of G4 and G2 greater than that of G1 (P<0.05), but no difference between G2 and G4. Blood glucose subsequently fell (P<0.01) during G2 and G4, but not G1. There was a dose-related suppression of the number of antral pressure waves (PWs) (P=0.05) *(Fig B).* Only G4 stimu-lated basal pyloric pressure (P<0.01), and none of the glucose infusions had any effect on isolated



pyloric or duodenal PWs. None of the glucose infusions had an effect on appetite perceptions, and only G4 decreased energy intake compared with C, G1 and G2 (P<0.05, *Fig C*). **Conclusion:** (i) The relationship between glycemia and ID glucose load is non-linear, (ii) the suppression of antral, but not pyloric, PWs appears to be load-dependent, and (iii) ID glucose loads ≤2 kcal/min do not appear to affect energy intake.

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Relationship between PYY and gastric satiation and motor functions in obesity

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Background: A previous report suggested that fasting and postprandial peptide-YY (PYY) levels are decreased in obesity and that PYY_{3-36} reduced appetite in obesity (NEJM 2003;349:941-8). The mechanism of the reduced PYY level in obesity is unclear. PYY is a mediator of the ileal brake mechanism, altering gastric motor function. **Hypothesis:** Delayed gastric emptying is associated with reduced PYY, and reduced PYY is associated with altered gastric motor function and satiation in obesity. **Aims:** To compare gastric motor and sensory functions and plasma fasting and postprandial PYY levels in healthy humans. **Methods:** We studied 71 participants in normal, overweight, or obese (WHO criteria) groups. All were non-bulimic on validated questionnaire and underwent the following measurements using validated tests: gastric emptying (GE) for solids and liquids by scintigraphy ($t_{1/2}$); gastric volumes (GV) by SPECT; maximum tolerated volume (MTV) by satiation test with Ensure® at 30 ml/min. Blood samples were collected fasting and postprandially for 2 hours. The univariate associations among measured responses were assessed using Spearman correlations. Multiple linear regression models adjusting for body weight and gender assessed these factors for their independent ability to predict postprandial PYY and MTV. **Results:** On univariate analysis: (a) GE $t_{1/2}$ for solids (r_s=-0.25; p=0.03) and liquids (r_s=−0.32; p=0.0065) were inversely correlated with postprandial PYY; (b) increased body weight was associated with faster GE (inverse relationship to solid GE $t_{1/2}$ (r_s= −0.33, p=0.005) and liquid GE $t_{1/2}$ (r_s= −0.24, p=0.04); (c) No significant correlation between body weight and fasting (r_s=0.18, p=0.13) or postprandial PYY levels (r_s=0.05, p=0.65). Multiple variable analysis indicated only liquid GE $t_{1/2}$ was a predictor of postprandial PYY (p=0.03). GV change after the meal and postprandial PYY were independent predictors of MTV (both p=0.01); together they accounted for 18% of the variance in MTV. **Conclusion:** Obesity is not associated with decreased PYY levels; faster liquid GE increases PYY levels. Satiation (MTV) is significantly influenced by the postprandial PYY and gastric volume, suggesting that PYY may mediate, in part, gastric motor and sensory function in obesity. Grant support: NIH RO1 DK67071 (MC)

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STW 5 (Iberogast®) evokes chloride secretion in human intestine

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The hydroethanolic drug STW5 consisting of nine herbal extracts (bitter candy tuft, chamomile flower, peppermint leaves, caraway fruit, liquorice root, lemon balm leaves, angelica root, greater celandine herbs, and milk thistle fruit) is successfully used to treat functional dyspepsia. Clinical data would suggest that it may be of therapeutic value for treating patients with irritable bowel syndrome (IBS). We therefore investigated the effect of STW5 on secretory activity of mucosa/submucosa preparations from human ileum and colon using the Ussing chamber technique. Experiments were performed on normal tissue from surgical specimen (61 preparations from 30 patients, age:69.8±11.1). Serosal application of STW5 (256μg/ml-1024μg/ml) concentration dependently increased the short circuit current in small and large intestine. The increase was 9.7±2.9μA/cm2 for 256μg/ml, 22±7.9μA/cm2 for 512μg/ml and 29±8.1μA/cm2 for 1024μg/ml (p<0.05 at all concentrations). The concentrations used are well below the dose used to treat patients which is 3x 51mg/ml daily. The STW5 evoked secretory effect was bumetanide (100μM) and glibenclamide sensitive and therefore due to increased chloride secretion. Nerve blockade by tetrodotoxin (1μM) had no effect indicating a direct epithelial action of the drug. Accordingly, STW 5 caused pronounced, bumetanide sensitive, secretory responses in the colonic epithelial cell line T84. In addition the secretory response after electrical field stimulation of nerves remained unchanged in the presence of STW5. Our results indicate that STW5 has a significant and sustained pro-secretory effect in the human intestine *in vitro*. It does not interfere with neurally mediated secretion but stimulates chloride secretion at the level of the epithelial cell. Generally, patients with reduced secretion may profit from this drug. STW5 (Iberogast®) may represent a novel treatment option for IBS patients, in particular those with constipation predominant IBS.

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Methylnaltrexone: An investigational drug to reverse opioid-induced GI hypomotility

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Methylnaltrexone (MNTX), a quaternary derivative of naltrexone, was developed to antagonize the peripheral adverse effects of opioids while preserving centrally mediated analgesia. MNTX is being developed for opioid-induced inhibition of GI motility in advanced illness (AI), chronic pain, and postoperative bowel dysfunction (POBD). In volunteer studies GI transit slowed by morphine (measured by oral-cecal transit time (OCTT)) was reversed by intravenous (IV), subcutaneous (SC), or oral MNTX. To study MNTX in chronic opioid users, a double-blind, placebo-controlled, randomized trial was performed in 22 subjects on chronic methadone maintenance. Laxation occurred within one minute of injection of IV MNTX in all subjects without inducing withdrawal. In a trial in volunteers, single oral doses of MNTX (ranging from 0.64 to 19.2 mg/kg) induced laxation within hours. Similar effects were seen with oral MNTX in 12 methadone maintenance subjects. After SC dosing, changes in OCTT occurred in about 20% of healthy subjects over a period of about 15 minutes. A phase 2b study of SC MNTX in 33 AI pts with opioid-induced constipation showed dose-related laxation. More than 70% of the treated pts laxated, most within one hour, without significant side effects or evidence of withdrawal. This was confirmed in a randomized double-blind placebo-controlled phase 3 trial, in which 154 pts with AI received either a single SC dose of MNTX (0.15 or 0.3 mg/kg) or placebo followed by 4 weeks of open label therapy: 61.7% laxated within 4 hrs of their first drug injection (0.15 mg/kg) vs. 13.5% with placebo (p<0.0001). In a second phase 3 study, 133 AI pts were treated with SC MNTX (0.15 mg/kg) or placebo qod for 2 weeks. Within 4 hrs of the first dose, 48.4% of MNTX pts vs. 15.5% of placebo pts achieved laxation (p<0.0001). Laxation within 4 hrs of dosing for at least two of the first four doses occurred in 51.6% of MNTX pts vs. 8.5% of placebo pts (p<0.0001). Importantly, responding pts laxated within a ½ hr of treatment after the first dose, allowing a measure of predictability in these complex pts. A recently reported randomized, double-blind, placebo-controlled phase 2 trial of IV MNTX in 65 pts undergoing colectomy demonstrated a 23 hr improvement in first laxation (p=0.01) and a 30 hr acceleration in eligibility for hospital discharge (p=0.03) in treated pts, suggesting a role in relieving POBD. MNTX was well tolerated in the above studies and has demonstrated the potential to reverse opioid induced hypomotility, rapidly and predictably in clinical settings such as opioid induced constipation and POBD without affecting analgesia.

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Effects of celecoxib and diclofenac on gastric motor and sensory function in healthy volunteers

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Introduction: Non-steriodal antiinflammatory drugs (NSAIDs) frequently cause dyspeptic symptoms unrelated to mucosal ulcerations. The tolerance profile of selective COX-2 inhibitors as compared to non-selective Cox inhibitors is still poorly understood. Furthermore the pathophysiology of NSAID-induced dyspeptic symptoms remains controversial. **Aims:** To compare gastric compliance, tonus and sensory function by gastric barostat in healthy volunteers after intake of diclofenac and celecoxib. **Methods:** Healthy volunteers (HV) were included in a randomized double-blind placebo-controlled crossover study. After endoscopy, abdominal US, and H.p. testing celecoxib (Cel) 200 mg qam + placebo qhs, diclofenac (Dic) 75 mg bid or placebo (Pla) bid were administered over 14 days. Each subject underwent two dosing periods separated by a 2 week wash-out period according to an factorial design. At the end of each dosing period subjects underwent gastric barostat testing assessing pre- and postprandial (300 kcal liquid meal, 200 ml) gastric motor and sensory function. **Results:** 12 HV (6f; age 32, range 20-49 years) completed the study. The perception threshold pressure was higher both for Cel and Dic versus Pla preprandially, and lower during Dic compared to Cel ($p<0.05$) postprandially. Postprandial gastric compliance was higher ($p<0.05$) during Cel compared to Dic. Gastric tonus in the pre- and postprandial periods was lower ($p<0.05$) during Dic compared with Pla and Cel. **Summary:** Celecoxib and diclofenac increase fasting perception thresholds. Postprandially diclofenac lowers perception thresholds compared to celecoxib. Diclofenac but not celecoxib impairs gastric adaptive relaxation. **Conclusions:** The more favorable side effect profile towards generation of dyspeptic symptoms of selective COX-2 inhibitors compared to non-selective Cox inhibitors may be in part explained by their effect on postprandial gastric visceral sensation and relaxation.

Mean pre- and postprandial values	Placebo	Diclofenac	Celecoxib
Threshold Pres (mmHg) pre/post	10.5[a,b] / 10.6	12.6[a] / 9.9[c]	12.4[b] / 11.2[c]
Compliance (ml/mmHg) pre/post	109/98	88/90[c]	118/110[c]
Tonus (ml) pre/post	202[a] / 364[a]	131[a,c] / 260[a]	193[c]/322

[a] Pla vs. Dic $p< 0.05$, [b] Pla vs. Cel $p< 0.05$, [c] Dic vs. Cel $p< 0.05$

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Protein and ginger for the treatment of chemotherapy-induced delayed nausea and gastric dysrhythmia

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Background: Delayed nausea occurs in many cancer patients during the three days following administration of a cytotoxic agent. Meals high in protein content decrease the nausea of motion sickness and pregnancy, possibly by reducing gastric dysrhythmias. Ginger may also have anti-nausea properties. The aim of this study was to explore the use of a nutritional intervention consisting of high protein meals and ginger for the management of delayed nausea experienced by chemotherapy-naïve cancer patients. **Method:** Twenty-eight cancer patients receiving emetogenic cytotoxic agents were assigned to one of three groups. During the three day study period following their first chemotherapy session, Control Group patients (n=9) continued with their normal diet, Protein Group patients (n=9) were provided with a protein drink (15 g protein) and 1 g of dried ginger root to consume twice daily, and High Protein Group patients (n=10) were provided with a protein drink with additional protein powder (31 g protein) and 1 g of dried ginger root to consume twice daily. All patients completed a symptom diary each day to assess the severity, frequency, and impact of the nausea they experienced, as well as their use of antiemetic medication. Gastric myoelectrical activity was assessed by electrogastrography as five patients ingested a protein meal with ginger on the first morning of the study. **Results:** Reports of nausea, of nausea being experienced often, and of nausea being bothersome were significantly less frequent in the High Protein Group than in the Control and Protein Groups ($ps<.05$); the Control and Protein Groups were not significantly different from each other. Furthermore, significantly fewer patients in the High Protein Group elected to use antiemetic medication than patients in the other two groups ($p<.05$). A significant increase in normal gastric myoelectrical activity, and a significant decrease in gastric tachyarrhythmia, the gastric dysrhythmia that frequently accompanies nausea, occurred with ingestion of the protein meals and ginger ($ps<.05$). **Conclusions:** High protein meals with ginger reduced the delayed nausea of chemotherapy, and reduced the use of antiemetic medications. The anti-nausea effects were associated with enhancement of normal gastric myoelectrical activity and decreased gastric dysrhythmias. High protein meals with ginger represent a novel, nutritionally-based treatment for the delayed nausea of chemotherapy.

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Acupuncture treatment, using acupoints P6, SP4 and DU20, is more effective for nausea than for bloating or pain in patients with severe functional nausea

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Acupuncture has been reported to improve symptoms in patients with nausea secondary to chemotherapy, post-operatively and in pregnancy. Its utility in functional bowel disorders is less clear. **Aim:** to determine the efficacy of acupuncture in treatment (Rx) of functional nausea, bloating and abdominal pain in patients refractory to prokinetics. **Methods:** 22 patients (pts) were referred for Rx. Charts were reviewed for severity of nausea, abdominal pain and bloating. Severity of each symptom was scored before and after each acupuncture Rx on a visual analog scale (VAS) with 0 = none and 100 = severe. 15 min of baseline EGG were recorded. At the first study pts underwent a water load test. Acupuncture points P6, SP4 and DU20 were needled for 30 min, while the EGG was recorded. Pts returned for one or two additional Rxs which were at least 2 weeks apart. EGG was analyzed to record the percent of power in the 1-2.5 cpm, 2.5-3.7 cpm, 3.7-10cpm and 10-15cpm frequency ranges at baseline and during each Rx. Pts with none or mild symptoms of nausea by history were analyzed together (MN, n=12) and those with severe nausea were analyzed together (SN, n=10). **Results:** Data are expressed at mean ± SEM. In both groups, most pts were women and had normal gastric emptying. No differences in mean weight or age were noted. Pts with SN tolerated a lower volume during the water load test (429±34 vs. 643±22, $p<0.0001$). At entry, the nausea score was greater in SN compared to MN (68±2 vs. 33± 2, $p<0.001$). Acupuncture decreased nausea scores with each Rx. In both groups, there was significant improvement in baseline nausea score between the first and third acupuncture treatments, $p<0.05$. Also, comparing nausea at entry to after the third Rx , scores improved from 33±2 to 7 ± 2, $p<0.001$ for MN, and from 68 ± 2 to 22 ± 8, $p <0.001$ for SN. In MN group, pain and bloating improved when comparing preRx to after 3rd Rx, from a pain score of 44 ±10 to 10 ± 6, $p<0.01$;and bloating score from 63 ± 10 to 32 ± 8, $p<0.03$. No improvement in pain or bloating was seen in the SN group. In both groups EGG data showed an increase in 2.5 –3.7cpm after treatment, $p<0.03$. **Conclusions:** Water load test data suggests a different sensory afferent pathophysiology in severe compared to mild nausea goups. Acupuncture achieved significant and persistent improvement in nausea. Bloating and pain improved only in MN group. The differential improvement in nausea compared to bloating or pain argues against a pure placebo effect. The acupoints used include points for both nausea and depression and a holistic approach to this condition may prove more effective than stimulation of P6 alone.

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Glutamate-induced calcium currents in neurons of the dorsal motor nucleus of the vagus nerve

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Purpose: To determine the mechanism of glutamate receptors involved in intracellular calcium signal in neurons of the dorsal motor nucleus of the vagus nerve (DMNV). **Methods:** 1) **In vitro culture of DMNV**. Neonatal Sprague-Dawley rats were sacrificed, the brainstem was removed, and the DMNV area was excised using a dissecting microscope. Cells were plated onto poly-d-lysine coated culture dishes. 2) **Measurement of Intracellular Calcium**. Single-cell cytoplasmic calcium ([Ca2+]) was determined from the ratio of fluorescence intensity of fura-2-AM at 340 and 380 nm. Cells were considered responsive if maximal change in fluorescence (ΔF/F0) was equal to or greater than 10% from the baseline for each experimental condition. **Results:** Exposure to glutamate (10 μm) for 60 seconds caused intracellular Ca^{2+} increments in greater than 80% of cells (n = 223). Glutamate produced dose-dependent increments in maximal $\Delta F/F_0$ and in the percentage of cells responding (n = 808, P < 0.05). Repetitive exposure to glutamate did not produce progressive decrements in peak $\Delta F/F_0$, suggesting that receptor-mediated mobilization of intracellular Ca^{2+} was not involved. This was confirmed when removal of extracellular Ca^{2+} abolished intracellular Ca^{2+} transients (n = 115). Kynurenic acid (1mm), a non-specific glutamate receptor antagonist, blocked intracellular Ca^{2+} transients in all cells (n = 81). Exposure to glutamate while blocking AMPA receptors with GYKI 52466 (50 μm) abolished the Ca^{2+} response in all cells (n = 43). Exposure to (S)AMPA (5 μm) for 60 seconds caused intracellular Ca^{2+} increments in 97% of cells (n = 78). Dizocilpine (10 μm), a non-competitive NMDA antagonist, abolished Ca^{2+} signal in only 1.4% of cells (n = 91). Exposure to NMDA (10 μm) produced no intracellular Ca^{2+} transients in any cell tested (n = 68). (2S,4R)-4-Methylglutamic acid (100 nm), a kainate receptor antagonist, did not abolish the Ca^{2+} response in any cells (n = 88). Exposure to the kainate receptor agonist, ATPA (10 μm), produced no intracellular Ca^{2+} transients in any cell (n = 129). NiCl (3mm), a non-specific Ca^{2+} channel blocker, abolished intracellular Ca^{2+} transients (n = 50). Blocking T-type Ca^{2+} channels with mibefradil (20 μm) abolished the Ca^{2+} response in 76% of cells (n = 68). Nifedipine (1 μm), an L-type Ca^{2+} channel blocker, and conotoxin (1 μm), an N-type blocker, did not abolish the Ca^{2+} response (n = 101 and 58, respectively). **Conclusion:** Glutamate mediates intracellular Ca^{2+} currents by binding to the AMPA receptor, which opens T-type Ca^{2+} channels allowing an influx of extracellular Ca^{2+}.

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GDNF promotes the survival of nNOS neurons through NPY

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Background and Significance: Glial Derived Neurotrophic Factor (GDNF) is a growth factor known to induce proliferation and suppress apoptosis in enteric neurons. Neuropeptide Y (NPY) is a peptide neurotransmitter shown to be a neuroproliferative factor in postnatal olfactory neurons. We investigated the role of NPY in GDNF mediated enteric neuronal proliferation and suppression of apoptosis. **Methods:** Using a primary culture system of enteric neurons obtained from E14.5 rat embryonic intestines by immunoselection, we examined the effect of GDNF on NPY mRNA (real time PCR) and protein expression (Immunostaining) and release of NPY into the culture medium (ELISA). Proliferation was assessed by BrdU incorporation and apoptosis assessed by the TUNEL method and immunocytochemistry for cleaved caspase-3. NPY-mediated phosphorylation of Akt was assessed by western blot analysis. For the *in vivo* studies segments of proximal colon obtained from WT and $NPY^{-/-}$ mice were fixed and stained for peripherin, NADPH diaphorase and ChAT (Choline acetyl transferase) to assess total number of myenteric neurons, inhibitory neurons and excitatory neurons respectively. Electric field stimulation (EFS) of proximal colon strips was used to evaluate the inhibitory neuronal mediated relaxation of the colon. **Results:** GDNF (24 h) induced a significant increase in NPY mRNA (P<0.0001), number of NPY expressing neurons (P<0.005) and release of NPY into the culture medium (P <0.002). GDNF did not induce the release of PYY, a peptide closely related to NPY. Exposure of GDNF or NPY (1μM, for 24h) significantly increased proliferation of enteric neurons (P<0.006) and reduced apoptosis in enteric neurons compared to controls (P<0.002). NPY induced a significant increase in $nNOS^+$ enteric neurons compared to vehicle treated cells (P<0.01), but no change in $ChAT^+$. NPY increased phosphorylation of Akt in a PI3-Kinase dependent fashion (P<0.05). In $NPY^{-/-}$ mice, there was a decrease in the number of NADPH diaphorase staining neurons in the colon (P<0.01), but not the $ChAT^+$ staining neurons. EFS evoked relaxation of the proximal colon was significantly impaired in $NPY^{-/-}$ mice compared to WT mice (% relaxation at 48V: WT: 40.94 ± 4.6, $NPY^{-/-}$: 15.27 ± 1.3, P=0.002, n=4). The neuronal staining as well as EFS data is consistent with a decrease in the inhibitory neurons in $NPY^{-/-}$ mice. **Conclusions:** We demonstrate a novel pathway of GDNF mediated NPY release with subsequent promotion of enteric neuronal proliferation and survival. NPY serves as a neuroproliferative factor for inhibitory enteric neurons and may be a potential target for modulating enteric neuronal proliferation and survival.

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Receptor operated channels (ROCs) in intestinal smooth muscle cells mediate Ca^{2+} influx induced by the activation of G protein coupled receptors signaling through a Gq protein pathway

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ROCs are members of the canonical transient receptor potential (TRPC) channel family and mediate a flux of cations following the activation of G-protein coupled receptors (GPCRs). GPCRs that activate phospholipase C (PLC) through a Gq-protein can modulate TRPC activity through either hydrolysis of phosphatidylinositol (4,5) bisphosphate (PIP_2), production of diacylglycerol (DAG), or production of inositol (1,4,5) triphosphate (IP_3) and release of Ca^{2+}. Ca^{2+} tightly regulates smooth muscle contraction and excitability. Activation of ROCs in intestinal smooth muscle might influence the Ca^{2+} homeostasis in these cells thus affecting gastrointestinal motility. The aim of this study was to investigate the signaling pathway involved in the activation of ROCs in cultured smooth muscle cells of the rat intestine using galanin 2-11, a peptide fragment that activates a Gq-mediated GPCR, galanin receptor 2 (GalR2). Smooth muscle cells were cultured from small intestine of 6-10 days old rats. $[Ca^{2+}]_i$ changes were monitored using the Ca^{2+} sensitive dye, fluo-3 with confocal microscopy and increasing concentrations of galanin 2-11 (0.01-1.0 μM) in the presence or absence of a PLC antagonist and of an inhibitor of Ca^{2+} influx mediated by ROC. Galanin 2-11 resulted in a concentration-dependent increase in $[Ca^{2+}]_i$, which was inhibited by the PLC antagonist, U-73122 (2 μM) indicating the involvement of a PLC pathway. Removal of extracellular Ca^{2+} abolished the galanin 2-11 mediated $[Ca^{2+}]_i$ increase, whereas it did not change Ca^{2+} release induced by ATP (10 μM). In addition, thapsigargin (1 μM), an agent that depletes intracellular Ca^{2+} stores, abolished both ATP and galanin 2-11-mediated $[Ca^{2+}]_i$ increase, suggesting that Ca^{2+} was not derived from intracellular stores. SKF-96365 (50 μM), an inhibitor of Ca^{2+} influx mediated by ROC, prevented the galanin 2-11 induced $[Ca^{2+}]_i$ increase supporting our hypothesis that Gq-mediated GPCR activation involves a plasma membrane channel. These results suggest that opening of a ROC through GPCR activation influences smooth muscle cell excitability thus modulating gastrointestinal motility. The ROC involved in the Gq/PLC-mediated GPCR activation is likely to be a member of the TRPC3/6/7 subfamily, which includes TRPC channels expressed in different smooth muscle cell types.

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The effect of the cholinergic anti-inflammatory pathway on experimental colitis

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Background and aim: Recent studies have reported that neuroimmune interaction can affect the process of intestinal inflammation. The cholinergic anti-inflammatory pathway is a neural mechanism that is controlled by the vagus nerve, and inhibits proinflammatory cytokine overproduction in inflammatory response. Inflammatory bowel diseases (IBD) are characterized by proinflammatory cytokines, tissue damage, and loss of neuron in inflamed mucosa, which implies the cholinergic anti-inflammatory pathway is destroyed during the process of inflammatory response. In the study, we studied the effect of cholinergic agonist as anabaseine and nicotinic receptor antagonist as chlorisondamine diiodide on TNBS-induced colitis, to investigate the potential therapeutic effect of the cholinergic anti-inflammatory pathway on IBD. **Methods:** Trinitrobenzene sulfonic acid (TNBS)-induced colitis was produced, 10μg anabaseine or 1.5μg chlorisondamine diiodide was administrated i.p to each mouse half an hour before the induction of colitis, and repeated on interval day till the mice were sacrificed. Inflammation was examined by histological analysis, myeloperoxidase (MPO) activity, and the production of proinflammatory cytokines as TNF-α, IL-1β in colonic tissue. The expression of neuronal marker as S100 protein and neurofilament protein was determined by immunohistochemistry. **Results:** The mice with colitis treated by anabaseine showed less tissue damage, less MPO activity, and less proinflammatory cytokine level, compared with those mice with colitis untreated, whereas the mice with colitis treated by chlorisondamine diiodide showed worst tissue damage, highest MPO activity, and highest proinflammatory cytokine level. The expression of S100 protein and neurofilament protein was absent in inflammatory mucosa of mice with TNBS-induced colitis or those treated by chlorisondamine diiodide. However, administration with anabaseine restored the expression of those neuronal markers in inflammatory mucosa of mice with TNBS-induced colitis, and protected the mucosal neuron file from loss. **Conclusion:** Our data shows the agonist of the cholinergic anti-inflammatory pathway inhibits colonic inflammatory response by down-regulating the production of proinflammatory cytokines, and inhibiting loss of mucosal neuron, which suggests that modulating the cholinergic anti-inflammatory pathway may be a new potential management for inflammatory bowel diseases.

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Limitations of a TCA's serotonergic and adrenergic effects on visceral nociception in a rat model

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Background: Tricyclic antidepressants (TCAs) like Amitriptyline are established drugs in the treatment of IBS. Lately modern 5-HT agonists have demonstrated the importance of serotonergic mechanisms on visceral perception. The heterogeneity of potential drug actions of TCA's obviously plays a crucial role in their efficacy on visceral pain. In contrary, SSRIs fail to show these effects. **Aims:** This study aims to characterize the influence of amitriptyline on visceral nociception. Reserpine was used to antagonize TCA's sertonergic actions in order to discriminate these effects from others. **Methods:** We performed colorectal distensions (CRD) with a barostat device in male Lewis rats and assessed the visceromotor response (VMR) to tonic distension by abdominal wall electromyography. 2 hours prior to CRD we either applied Amitriptyline (15mg/kg) or Reserpine (1.5mg/kg) singularly or in coadministration intraperitoneally (i.p.). Controls received saline i.p. Serotonin (5-HT) serum levels were determined by HPLC protocol. **Results:** Administration of Amitriptyline caused a significant decrease in VMR compared to controls [area under the curve (AUC) to CRD in controls was 5841μV(±2438μV), in 15mg/kg: 3766μV (±1626μV;$p<0.05$)]. In rats pretreated with Reserpine the AUC under CRD was 7359μV(±1418μV;$p<0.001$). Coadministration of both drugs resulted in an AUC of 6329μV(±1158μV). 5-HT levels were increased in amitriptyline pretreated rats compared to controls. Significantly lower 5-HT levels were found in rats pretreated with reserpine as well as in the combination of reserpine+TCA. **Conclusions:** Amitriptyline has a significant antinociceptive effect, elevating the 5-HT serum levels. Administration of Reserpine in contrary resulted in a significant increase of the VMR, correlating with low 5-HT serum levels. Coadministration of both drugs attenuated Reserpine's effects on VMR, without any effect on the low 5-HT levels. This could be explained by TCA's additional effects on visceral nociception. Serotonergic effects seem to play an important role in TCA's mode of action on attenuation of visceral nociception but anticholinergic effects or presumably direct antinociceptive effects of these drugs should not be underestimated and are subject of further investigations.

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Reflex movement of the intestine is a major cause of 5-HT release from enterochromaffin cells

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Introduction: The release of 5-HT from the enterochromaffin (EC) cell is one of the critical steps involved in sensory transduction from the intestinal lumen to the sensory neurons. What is unclear is the contribution 5-HT makes to ongoing enteric reflexes and how the moment-to-moment release of 5-HT is controlled. The aim of this study was to determine if motility reflexes interact with the intrinsic mechanosensitivity of the EC cell and subsequently cause 5-HT release. **Methods:** Guinea pigs (Hartley) were anesthetized with isoflurane, then killed by severing the carotid arteries and spinal cord. Full thickness ileum was loosely pinned, mucosa up, in a bath of warmed physiological saline. Electrochemical recordings were made using 7 μm carbon fiber electrodes and the tension in the circular muscle was recorded with a force transducer. The concentration of 5-HT was calculated from the current at +350-450 mV and the current produced by exogenous 5-HT (5 μM). **Results:** Stretch-evoked motility reflexes caused 5-HT release and this release was not reduced by atropine (muscarinic receptor antagonist; n=3). In contrast, release was significantly reduced by the smooth muscle relaxants nitroprusside, isoproterenol or papaverine (n=5 for all). Addition of atropine to any of these relaxants caused complete quieting of the smooth muscle and block of stretch-evoked 5-HT release (n=5 for all). Compression evoked 5-HT release was unaffected by these treatments. In order to exclude the possibility that contact with the mucosa by the electrode contributed to the motility induced 5-HT release, the electrode was positioned 100-200 μm above the mucosa. Under these conditions the concentration of 5-HT detected was ~10-fold less, but was identical in time course (n=3). **Conclusion:** Stretch-evoked 5-HT release is the result of mechanical stimulation of the EC cells by the movement of the underlying musculature. Thus, mechanical stimulation as a consequence of the activation of motility reflexes contributes significantly to physiological 5-HT release. **Support:** Department of Physiology & Cell Biology, University of Nevada, School of Medicine.

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CCK-1 receptors couple to TRPC - like channels in GI nodose ganglion neurons (NGN) and transfected HEK-293 cells

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CCK induces satiety through acting on CCK-1 receptors on vagal afferents. Mechanism by which CCK stimulates gastrointestinal vagal afferents is unclear. TRPC channels are receptor operated channels and are thus potential candidates for mediating CCK induced responses. **Methods:** NGNs innervating the GI tract were labelled by i.p. injection of fast blue (4%, 10μL). 2-7 days later cells were isolated and

whole cell patch clamp recordings were performed on labelled neurons 12-48 hours later. HEK-293 cells were transiently transfected with plasmids encoding the CCK-1 receptor, the TRPC 5 channel or both. Cells were voltage clamped at -60mV and drugs applied using a fast flow solution switching system. Antagonists were applied 2-5 min prior to agonists. **Results:** CCK-8S (1-100nM) induced a concentration dependent, inward current in 38/72 (53%) of labelled NGNs. The amplitude of this current ranged from 10 – 150 pA (mean 38±4.5 pA), and the reversal potential was approximately +10mV. CCK induced currents were blocked completely and reversibly by the CCK-1 receptor antagonist lorglumide (1μM n=5). The effect of a number of putative TRP channel blockers on the CCK induced current was examined. Ruthenium red (3μM) (n=5) and 2-APB (100μM) (n=5) blocked the CCK induced current. Furthermore, in all CCK sensitive NGNs (n=12), and in 24/29 NGNs tested in total GdCl3 (100μM) activated a transient inward current. This current was completely and reversibly blocked by 100uM 2-APB (n=5). Prolonged application (3-5 min) of Gd desensitized the Gd sensitive response as well as the CCK induced current (n=10). In TRPC5 transfected cells, ATP (via P2Y receptors) activated an inward current in 10/14 cells however CCK had no effect. In CCCK-1R transfected cells, neither CCK nor ATP induced an inward current (n=10). In CCK-1R/TRPC5 cotransfected HEK cells, CCK induced an inward current in 11/15 cells, all CCK responsive cells also responded to ATP. The CCK induced current was completely blocked by lorglumide 1μM. **Conclusions:** CCK-1 receptors activate a TRP – like cation conductance in GI – projecting nodose ganglion neurons. All of these cells also express a GdCl3 activated TRPC4/5 – like current. In a heterologous expression system, CCK activates an inward current in cells expressing the CCK-1R and TRPC5. These results suggest that activation of TRPC channels is a major mechanism by which CCK activates vagal afferent neurons.

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Brain-derived neurotrophic factor (BDNF) enhances serotonin and substance P induced Ca^{2+} signaling and promotes vesicle recycling in cultured myenteric neurons

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Introduction: Brain-derived neurotrophic factor (BDNF) enhances motility in several species and has been implicated in the control of peristalsis (Grider, 2006). Besides being a potent modulator of neuronal activity and synaptic transmission, BDNF also has acute stimulatory effects on hippocampal neurons. Our aim was to study the mechanisms underlying BDNF's effect on motility by investigating 1) its acute effects on Ca^{2+} signaling of myenteric neurons; 2) the modulatory long-term effect on the signal transduction of two neurotransmitters serotonin (5-HT) and substance P (SP) and 3) its influence on presynaptic vesicle recycling. **Methods:** We used primary cultures of guinea pig myenteric neurons, which we loaded with Fluo-4 AM to monitor the intracellular calcium concentration ($[Ca^{2+}]_i$) changes. Neurons were identified by K^+ depolarization and $[Ca^{2+}]_i$ rises upon receptor stimulation were expressed relative to the K^+-evoked peak. To study efficiency of presynaptic vesicle turnover we used FM1-43. All signals were analyzed using Igor Pro®. **Results:** Immunohistochemistry showed that both BDNF and its high affinity receptor (TrkB) were expressed in the cultures. None of the neurons studied (n=90, N=10) displayed a $[Ca^{2+}]_i$ change when challenged with BDNF (50 ng/ml, 10s). However, after longer BDNF incubation (2, 24 or 48 hours, 50 ng/ml), the percentage of neurons responding to 5-HT (10^{-5}M; 10s) increased (92.2%, 91.7% and 91.0% respectively, χ^2, p<0.05 vs. control: 74.2%) and the $[Ca^{2+}]_i$ peak rose gradually with BDNF exposure (57.6 ± 0.8%, 63.3 ± 1.1%, 68.3 ± 1.0% and 72.9 ± 0.9% for control, 2, 24 and 48 hours respectively, ANOVA, p<0.05, n=256, N=11). Similarly, 24 and 48 hours of BDNF caused increased Ca^{2+} transients to SP (10^{-6}M; 10s, 68.5 ± 0.9% and 67.0 ± 0.7% respectively) compared to control (61.6 ± 0.6%, ANOVA, p<0.05, n=215, N=9). Although the percentage of SP responding neurons did not change, the proportion that displayed a second Ca^{2+} transient within 80 seconds after stimulus onset increased after BDNF incubation (53.7% and 55.1% for 24 and 48 hr respectively, χ^2, p<0.05 vs. control: 30.2%). Apart from this postsynaptic modulatory effect, we also found, using FM1-43, that BDNF (48 hr incubation) boosts the proportion of vesicles residing in the readily releasable pool, hence increasing synaptic efficacy. **Conclusion:** Unlike in hippocampal neurons, BDNF does not induce $[Ca^{2+}]_i$ changes in myenteric neurons directly, but increases 5-HT and SP induced Ca^{2+} signaling and promotes vesicle recycling efficacy, thereby enhancing ENS signaling both at the pre- and postsynaptic level. **Support:** FWO, Belgium.

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Piperine, a non-vanilloid congener of capsaicin, does it act solely via the capsaicin-sensitive sensory neurons in the mouse esophagus?

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A substantial number of primary sensory afferents in the mammalian esophagus, mainly of spinal origin, were shown to be immunoreactive to the transient receptor potential ion channel of the vanilloid type 1 (TRPV1) and called capsaicin-sensitive sensory neurons. We have shown that capsaicin can modulate the vagally induced esophageal peristalsis via a local neuronal reflex arc composed of capsaicin-sensitive sensory neurons and nitrergic myenteric neurons. Piperine, a non-vaniloid congener of capsaicin, is thought to produce its action through the activation of TRPV1. In a recent study, piperine, when compared with capsaicin, was shown to be less potent in activating the TRPV1 receptor but more efficient in term of its desensitizing ability. The aim of the current study was to investigate whether piperine can inhibit the vagally induced esophageal twitch contractions via its action on TRPV1 solely or it has other additional pathways. For this purpose, a thoracic esophageal segment including vagus nerves was dissected from adult ddY mice and placed in an organ bath. Piperine, like capsaicin, inhibited the vagally evoked contractions in a dose-dependent manner. Ruthenium red and SB-366791 antagonists of TRPV1 blocked the inhibitory effect of capsaicin but not piperine. NK1 receptor blocker, L-732,138, and nitric oxide synthase (NOS) inhibitor, L-NAME, blocked the inhibitory effect of capsaicin. However, these antagonists did not block the effect of piperine. After *in vitro* pretreatment of the esophageal preparation with capsaicin or piperine, capsaicin failed to produce its inhibitory effect, probably due to desensitization of TRPV1. Interestingly, the inhibitory effect of piperine can be reproduced even after *in vitro* pretreatment with capsaicin or piperine. Additionally, piperine, but not capsaicin, inhibited the vagally induced contractions in the esophagi of mice neonatally injected with capsaicin. In conclusion, the results of present study suggest that (a) piperine can influence the vagally mediated striated muscle contraction in mouse esophagus and that (b) the effect of capsaicin and piperine is triggered through common, probably capsaicin-sensitive sensory neurons, but also additional still unknown pathways for piperine. **Acknowledgements** This work was supported by a travel grant to J.W. from Kanehara Ichiro Foundation in Tokyo, Japan.

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Roles of excitatory and inhibitory neural pathways in spontaneous motor complex formation in guinea-pig colon

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The empty colon of several species shows a neural pattern of spontaneous motor activity consisting of circular muscle contractions slowly migrating in aboral direction. This motor pattern, named migrating motor complex, has been deeply investigated in the mouse. On the contrary, little is known about it in the guinea-pig. This study aimed to investigate the effects of excitatory or inhibitory neural pathway blockade on *in vitro* spontaneous motor activity of guinea-pig colon. Segments of guinea-pig distal colon were mounted under isotonic conditions inside 5-ml organ baths containing warmed (37° C) and gassed (O_2/CO_2 95/5%) Krebs solution. Colon preparations showed a spontaneous contractile activity consisting of periodic motor complexes. During a 30-min control period, that followed an 1-h equili-

bration period, maximal amplitude, duration, area under the curve (AUC) and mean period of motor complexes were 2.47±0.31 mm, 45.6±2.9 s, 50.2±8.0 mm s and 52.5±3.7 s, respectively (mean±s.e.m., n=7). Atropine (1 μM) reduced basal tone of segments and significantly increased the period of the motor pattern (to 75.8±3.9 s, P<0.001 vs. controls, one-way ANOVA, n=7). The addition of the selective NK_1 receptor antagonist MEN 11,467 (30 nM) to atropine did not significantly modify any of the measured parameters of the motor pattern (n=7). On the contrary, the addition of the selective NK_2 receptor antagonist nepadutant (1 μM) to atropine (1 μM) and MEN 11,467 (30 nM) further decreased circular smooth muscle tone and significantly increased the period (to 98.8±5.5 s, P<0.001 vs. atropine, one-way ANOVA, n=7) and decreased amplitude and AUC of the motor pattern (respectively, to 2.18±0.36 mm and 40.5±6.8 mm s, P<0.01 vs. atropine and P<0.01 vs. controls, n=7). In the presence of TTX (1 μM), smooth muscle tone increased and a new motor pattern appeared. The values of parameters of TTX-induced motor activity were: maximal amplitude, 1.1±0.2 mm; duration, 4.9±0.5 s; AUC, 2.7±0.9 mm s; mean period, 5.3±0.4 s (n=4). L-NAME (1 mM) or apamin (0.1 μM) increased smooth muscle tone and disrupted the periodic motor complexes (n=7 and n=4, respectively). These findings suggest that in guinea-pig distal colon the excitatory neurotransmission to circular smooth muscle contributes to basal muscle tone and regulates the frequency of spontaneous motor complexes. The inhibitory neurotransmission to circular smooth muscle is continuously active. The consequent basal muscle relaxation is fundamental for motor complex formation. (Supported by Fondi Ateneo 2006).

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5-HT_7 receptor mRNA expression in different regions of the guinea-pig gastrointestinal tract and its role in distal colon propulsion

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The effects of serotonin (5-HT) in the gastrointestinal tract are mediated by several receptor types (5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_7). The 5-HT_7 receptor has been found to relax smooth muscle and participate in the accommodation of the circular muscle during the preparatory phase of ileal peristalsis in the guinea-pig. Our aims were to measure by quantitative real-time PCR the mRNA amounts of the 5-$_{HT_7}$ receptors in various compliant organs and define its role in colonic propulsion. Gastric fundus and antrum, jejunum, distal ileum, caecum, proximal and distal colon of male albino guinea-pigs were used. Total RNA was isolated by the RNeasy Protect Mini Kit (Qiagen). First-strand cDNA was synthesized from the total RNA by the ThermoScript RT-PCR system (Invitrogen). Real time RT-PCR experiments were performed by the FastStart SYBR Green Master kit (Roche Applied Science). For the absolute quantification of 5-HT_7 mRNA, standard curves were constructed using different concentrations of standard 5-HT_7 DNA templates prepared by reamplifying products obtained from qualitative PCR. In functional studies, the velocity of an intraluminally distended balloon was considered as the main parameter of propulsive activity. A selective 5-HT_7 receptor antagonist (SB-269970: 100 nM) was used to determine the involvement of this receptor in peristalsis. PCR products corresponding to 5-HT_7 mRNA were found in all tissues examined by RT-PCR. Real time RT-PCR showed differences in the 5-HT_7 receptor mRNA amounts: the highest density was found in distal ileum (1.9 pg) followed by caecum (1.6 pg), jejunum (598.3 fg), gastric antrum (100.7 fg), proximal (54.9 fg) and distal colon (8.0 fg) and gastric fundus (4.79 fg) fragments. In functional studies, propulsion was reduced by approximately 50% by 5-HT_7 receptor blockade and was invariably blocked by the simultaneous administration of 5-HT_3, 5-HT_4 and 5-HT_7 receptors antagonists. 5-HT_7 receptor mRNA is present in variable amounts along the entire guinea-pig gastrointestinal tract, with the highest density in the distal ileum and caecum, two regions with reservoir function. In the guinea-pig distal colon, endogenous 5-HT plays a key role in the modulation of propulsion by activating 5-HT_3, 5-HT_4 and 5-HT_7 receptors, whose simultaneous blockade suppresses propulsive activity.

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Expressed porcine 5-HT_4 receptor splice variants: Do they resemble their human counterparts?

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Introduction: Ten human (a-i; n), three rat (a, b, e) and four mouse (a, b, e, f) 5-HT_4 receptor splice variants have been described. They all diverge in their C-terminal sequence except for the 5-HT_{4h} variant which has an insertion of 14 amino acids in its second extracellular loop. Although several functional differences between 5-HT_4 receptor splice variants have been described in heterologous expression systems, the physiological relevance of the different variants is still unclear. In spite of the pig being considered to be a relevant species to study human atrial 5-HT_4 receptor interactions as well as for studying human digestive function, no porcine splice variants have been described besides of the 5-HT_{4b} and 5-HT_{4h} variants. For the pig to be a good model for human gastro-intestinal (GI) and cardiac 5-HT_4 receptor behavior, however, tissue distribution and/or functionality of porcine 5-HT_4 receptor splice variants should be similar to human. The aim of this study was therefore to search for the occurrence and tissue distribution of additional 5-HT_4 receptor splice variants in the pig. **Methods:** Towards this aim, a rapid amplification of cDNA ends (RACE) approach was applied using total RNA isolated from the mucosal and muscular fraction of the proximal stomach and the adrenal gland of newborn pigs (2,3 days) as well as from the left and right atrium of newborn and young pigs (25 kg). 3'-RACE-ready cDNA was generated and used in a nested PCR approach to amplify 5-HT_4 receptor-specific cDNAs, using 5-HT_4-specific forward primers. The resulting PCR products were size-fractionated on an agarose gel, excised, purified, cloned into a bacterial vector and sequenced. Tissue distribution (GI tract, atrial, adrenal tissues) of the identified splice variants, in addition to the 5-HT_{4h} variant, was assessed using RT-PCR. **Results:** cDNA clones corresponding to the human 5-HT_{4a} (with large heterogeneity in the 3′ untranslated region) and 5-HT_{4b} variant were identified in porcine tissues, while none of the other human variants was found. Additionally we found nine splice variants that have not been described previously. Expression of some variants appeared to be tissue-related. The 5-HT_{4h} variant was found to be preferentially expressed in the mucosal fractions of the gastrointestinal tissues, while in the muscle layer the variants that lack the h-exon predominate. Further studies to confirm the functionality of some of the newly identified variants are ongoing. In conclusion, we identified eleven porcine 5-HT_4 receptor splice variants, nine of which have not been described in man.

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Obestatin is not involved in the regulation of food intake and gastric emptying in rodents

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Background and aim Obestatin has recently been discovered in the rat stomach. Obestatin is encoded by the ghrelin gene and is located downstream to ghrelin in the ghrelin precursor. It has been claimed by Zhang et al. (Science 310: 996, 2005) that obestatin behaves as a physiological opponent of ghrelin and inhibits food intake, gastric emptying and jejunal contractility. Obestatin has also been claimed to be the natural ligand of the GPR39 receptor, but this could not be confirmed by others (T.Schwartz, Keystone Symposium 2006). Still, in GPR39 knockout mice, gastric emptying is accelerated (Depoortere et al., Gastroenterology 130:A104, 2006). The aim of the present study was to verify the effects of obestatin on food intake, gastric emptying and intestinal contractility in rodents. **Methods** The effect of obestatin (125 nmol/kg) on 6-hour cumulative food intake was studied in

mice fasted for 19h. Gastric emptying was measured with the ^{14}C octanoic breath test in mice. Fasted mice (n=12) were injected i.p. with either saline, or increasing doses (60-250 nmol/kg) of obestatin 15 min before eating a meal of chow and baked egg yolk, doped with 0.5 μCi ^{14}C-octanoic acid. Excreted $^{14}CO_2$ was captured during 4h, counted and gastric half excretion time (t_{half}) and t_{lag} were calculated. In vitro, the effect of obestatin (10^{-6} M) was studied on electrically stimulated and non-stimulated strips from the fundus and small intestine of mice and rats suspended in a tissue bath. **Results** Obestatin at 125 nmol/kg did not affect reflex hyperphagia. The cumulated food intake (6 h) amounted 2.33±0.22 g after injection of obestatin and was not significantly different from the amount ingested after injection of saline (2.30±0.20g). Obestatin did not affect gastric emptying parameters in mice. Gastric half excretion time (t_{half}) after injection of saline was 71±4 min and was not significantly changed after administration of obestatin at 60 nmol/kg (69±4 min), 125 nmol/kg (82±10 min) or 250 nmol/kg (67±6 min). T_{lag} values were also not affected. Mouse intestinal and fundic smooth muscle strips did not respond to obestatin neither in the absence nor in the presence of electrical field stimulation. Negative results were also obtained with intestinal and fundic strips from rats. **Conclusion** At the doses tested, obestatin does not affect food intake, gastric emptying and intestinal contractility. Our results suggest that obestatin does not play a key role in the regulation of energy homeostasis and gastric emptying and do not support the concept that obestatin is a physiological opponent of ghrelin.

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Physiological significance of small heat shock proteins in contraction/relaxation of colonic smooth muscle

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Background: In smooth muscle, PKC-mediated phosphorylation of HSP27 is associated with contraction while cyclic nucleotide kinase (PKA or PKG) mediated phosphorylation of HSP20 on ser16 is associated with relaxation. Objective: To determine the effect of activation of contractile or relaxant pathways on the association of HSP27 with HSP20; and their respective translocation in or out of caveolin containing lipid raft. **Methods:** 1. HSP20 phosphorylation was determined by isoelectric focusing using cultured cells treated with 8-bromo-cAMP (8-Br-cAMP) or acetylcholine (Ach). 2. Detergent-resistant membrane rafts were isolated by sucrose gradient centrifugation and were analyzed by immunoblotting. **Results:** 1. Ach-induced a quick and significant phosphorylation of HSP20 within 1 min; while phosphorylation induced by 8-Br cAMP was much slower (within 30 min) and of much lesser magnitude (10 fold). 2. Ach induced a sustained and increased association of HSP27 with HSP20 in the particulate fraction, while this association decreased upon activation of PKG (SNP). 3. In resting cells, HSP20 was present in the caveolin-containing lipid raft fractions. Upon Ach-induced contraction, HSP20 translocated out of lipid raft fractions containing caveolin. 4. In resting cells, HSP27 was absent from the caveolin-containing lipid raft fractions. Following Ach-induced contraction, HSP27 was present in the caveolin-containing lipid raft fractions. Summary: Ach-induced contraction was associated with: 1) a significant and rapid substantial phosphorylation of HSP20; compared to slow and low level phosphorylation of HSP20 associated with relaxation induced 8-Br-cAMP; 2) an increased association of HSP20 with HSP27 in the particulate fraction of CSMC with no change in association upon relaxation by SNP; 3) In resting cells, HSP20 was present within the caveolin-containing lipid raft while Ach-stimulation (contraction) induced translocation of HSP20 out of these lipid raft microdomains. The translocation of HSP20 out of lipid raft fractions was kinetically coupled to its phosphorylation by Ach, and 4) HSP27 translocated into the lipid raft fractions in response to Ach stimulation. **Conclusion:** We have identified an apparent molecular switch between contraction and relaxation, regulated by the small heat shock proteins HSP20 and HSP27 in association with caveolin-containing lipid raft microdomains. In relaxed cells, HSP20 is present in the caveolin containing lipid rafts. Ach-induced contraction results in phosphorylated HSP20 moving out while phosphorylated HSP27 moving into the caveolin containing lipid rafts microdomains.

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Localisation of muscarinic receptor M1-M3 immunoreactivity in human colon

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Physiological, pharmacological, radioligand binding and *in-situ* hybridisation studies show human intestine has M1, M2 and M3 muscarinic receptor subtypes, but the precise localisation of receptor subtypes is not known due to lack of specific antibodies for immunhistological studies. This study used new antibodies, fluorescence immunohistochemistry and confocal microscopy to determine the precise cellular location of cholinergic muscarinic receptor subtypes M1-M3 in human colon. **Methods:** Mid transverse colon removed from children with familial adenomatous polyposis was fixed, frozen, sectioned and incubated with M1, M2 or M3 antisera followed by fluorescent secondary antibodies. Sections were double labelled with antibodies against synaptophysin to display nerve varicosities and cKit to identify interstitial cells of Cajal. Images were collected on a Leica LSM confocal microscope to allow the subcellular location of receptors to be defined. Preabsorption with the immunising peptide and western blotting were used to confirm antibody specificity. Colon was also snap frozen, RNA extracted and RT-PCR performed to confirm expression of all receptor subtypes. **Results:** In paediatric human colon, mRNA was abundant for all 3 receptors (M1-M3). M1-immunoreactivity (-IR) was present in myenteric and submucosal neuron cell bodies and in endothelial cells of submucosal blood vessels. M2-IR was present on the surface of smooth muscle cells in both muscle layers and in varicosities on nerve fibres in myenteric ganglia and in the circular muscle. Strong M2-IR was present on endothelial cells in submucosal and serosal blood vessels. M3-IR was present on smooth muscle cells in both muscle layers, at lower abundance than M2. M3-IR was also present in some myenteric neuron cell bodies. Immunoreactivity was absent following preabsorption with immunising peptide. Western blotting confirmed the antibodies bound to proteins of the expected size. **Conclusion:** Consistent with pharmacological studies M2-IR and M3-IR were present on muscle with M2 more abundant than M3. Nerves contained all 3 receptors with different distributions. M1-IR was present in cell bodies of many myenteric and submucosal neurons suggesting that M1 receptors mediate cholinergic neuro-neuronal transmission in human colon. M3 was present in only a small subset of myenteric neurons. M2-IR was located presynaptically on nerve fibres suggesting that M2 receptors act as autoreceptors regulating acetylcholine release. M1-IR and M2-IR were present on endothelial cells in blood vessels suggesting they mediate cholinergic regulated vaso-activity.

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Glucagon-like peptide-1 activates gastric vagal afferent nerves

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Background: It has been proposed that the vagus nerve plays a role in mediating the effect of GLP-1 on gastrointestinal motility and feeding behavior. **Methods:** To obtain electrophysiological and molecular evidence for the contribution of the afferent pathway in chemoreception, afferent mass activity in the ventral gastric branch of the vagus nerve and receptor gene expression of GLP-1 in nodose ganglion were examined in 52 Sprague-Dawley rats. **Results:** Intravenous administration of GLP-1 at the doses 30-1000 pmol kg^{-1}, reaching physiological plasma concentrations, increased vagal afferent mass activity with the peak effect (13-52% above basal level, $p<0.05$) 3-5 min after the injection. Repeated administration of GLP-1 (1000 pmol kg^{-1}, five times at

15 min intervals) each elicited similar responses, about 50% increase of vagal afferent activity ($p < 0.05$). Injection of the GLP-1 antagonist exendin (9-39)amide (500 pmol kg^{-1}) 10 min before the administration of GLP-1 abolished the GLP-1 response at doses 30-300 pmol kg^{-1}. Exendin (9-39)amide alone had no effect on the response of gastric vagal afferents to repeated gastric loads with saline. As a control GLP-2 had no effect of vagal afferent mass activity. Vagal chemoreception of GLP-1 is further supported by expression of the GLP-1 receptor gene in the nodose ganglia, where also the GLP-2 receptor was used as a control. **Conclusion:** Peripherally administered GLP-1 at physiological plasma levels activates gastric vagal afferents, with no evidence of desensitization. This effect is blocked by the specific antagonist exendin(9-39)amide, suggesting that GLP-1 receptors in vagal afferent nerves mediate sensory input from the gastrointestinal tract.

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The initiation of EEC-transcription by neural signals in GI-mucosa

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Congenital diseases (e.g. Hirschsprung d.) as well as denervation experiments in rat intestinal mucosa have verified numerical and spatialmutations of 5-HTi.r.cells; I-cells,CCK i.r.; D-cells, Som.i.r.; N-cells, Neurot.i.r.;L-cells, Glic.i.r.; L-cells, Glu-cag.i.r. Signal transduction from the G-protein coupled crypt cell receptors to the nuclear DNA binding protein alters the gene activation on the cAMP-dependent activators/coactivators (e.g.CREB/CBP, highly conserved) together with the synergistic cooperating Q2 domain,which is associated with the DNA TATA-box TFIID (hTAF-II130). The processes utilize ATP and lead to phosphorylation. Particularly the general transcription factor TFIID constitutes the first step in the transcription initiation of the RNA polymerase II and it contains the sequence specific DNA activities for recognition of class II promoters. Contacts to the nucleosomal histones (H2A, H2B,H3, H4, H1) with acetylation of specific resides in the NH_2 termini by sub-complexes of TFIID in particular TAFII250,which contains like various coactivators and chromatin remodeling factors, multiple enzymatic activities like kinases, histone-acetyltransferases, bromodomain motifs,ubiquinitation e.g.the latter may be responsible for the switch of the positive ~ negative translation events e.g.in CREB/CBP activation with Kix and Kid by bromodomains, or by myc/max in the HIH/lZIP DNA-e-box during the terminal differentiation of the I-cells. From these observations we conclude the primary target for the neural signal to the GI mucoa is the basal DNA apparatus with its preinitiation complex (PIC), which then activates chromatin remodeling.

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Evidence that increased expression and function of the nociceptive vanilloid receptor 1 (VR1) is associated with inhibition of mTOR, activation of autophagy and cell injury in DRG neurons in diabetic sensory neuropathy

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Background: The capsaicin-sensitive VR1 receptor is a non-specific cation channel activated by heat, protons and endogenous ligands, and undergoes differential increase in expression and function in dorsal root ganglia (DRG) neurons in diabetic peripheral neuropathy. We hypothesized that activation of VR1 receptor induces both the autophagy pathway and programmed cell death (PCD) in DRG neurons in diabetic rats. Autophagy involves engulfment of injured organelles including mitochondria in autophagosomes and is regulated by mammalian target of rapamycin (mTOR). Autophagy has been implicated in both cytoprotection and PCD. **Methods:** Diabetes mellitus was induced in male Sprague-Dawley rats by streptozotocin (45 mg/Kg). Lower thoracic and lumbar DRGs from control and diabetic (1-3 months) rats were isolated, fixed and sliced or acutely dissociated and incubated with the VR1 receptor agonist capsaicin (10 μM) for 1 hour in the presence or absence of the 3-MA (10 mM; inhibits autophagy) or rapamycin (100 nM; inhibits mTOR and activates autophagy). Immunohistochemitry and Western blot analysis were performed to examine alterations of the following markers: VR1, p70 S6K1 (downstream target directly phosphorylated by mTOR, NF200 (marker for large DRG neurons), peripherin (marker of small DRG neurons), LC3 (marker for autophagosomes), caspase-activated DNase (CAD; marker for apoptosis), and μ-calpain (marker for oncosis). Age-matched healthy rats served as controls. **Results:** DRG neurons from diabetic rats displayed a significant differential increase in the levels of VR1 compared to control rats ($n=5$, $p<0.05$) in large DRG neurons associated with a 25±3% loss in this subpopulation of neurons in diabetic rats. Capsaicin treatment of isolated DRGs from diabetic rats but not controls resulted in a significant decrease in phosphorylation of p70 S6K1 and this down-regulation was enhanced in the presence of rapamycin. The levels of μ-calpain and CAD in response to capsaicin treatment were differentially increased in DRGs from diabetic rats compared to controls. Expression of LC3-II was also significantly increased in DRGs from diabetic rats after treatment with capsaicin. Treatment with the 3-MA normalized the levels of LC3-II and CAD. **Conclusion:** VR1 expression is increased in large DRG neurons and associated with inhibition of mTOR, activation of autophagy and PCD. Suppression of mTOR and subsequent induction of autophagy may underlie one of the mechanisms by which activation of VR1 induces programmed cell death in diabetic neuropathy.

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Nitrergic and Purinergic co-transmission: complementary mechanisms of relaxation in the human colon

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A co-transmission between ATP (trough $P2Y_1$ receptors) and NO is responsible of smooth muscle relaxation in the human colon. The aim of this work is to investigate the mechanisms involved in this co-transmission in order to analyze if both pathways are redundant or complementary causing relaxation. Muscle bath and microelectrode techniques were performed with strips obtained from surgical specimens for rectal neoplasm. Two single pulses IJPs were performed at different time intervals (from 1 to 15s). The response consisted of a fast IJP1 elicited by the first pulse followed by a second IJP2 elicited by the second pulse. The ratio IJP2/IJP1 was plotted vs time interval between pulses. Data were fitted with an exponential curve (from 0 to 1) using a non-linear regression. About 2.5 s after the first pulse the amplitude of the IJP2 was 50% of the IJP1 (ratio 0.5) and 80-90% was achieved with intervals from 5 to 8s after the first pulse. This result was similar both in the circular (n=6) and longitudinal muscle layers (n=5). Moreover the mechanism was independent of the amplitude of the first IJP (10, 20, 30mV n=6). Trains of 5 seconds (n=6) were performed at 1Hz (5 pulses) and 5Hz (25 pulses). At 1 Hz (5 pulses), the response consisted on a first fast IJP followed by non-response in the second pulse and smaller fast IJPs in the other three pulses. The response was abolished by MRS2179 10μM. In contrast at 5Hz a fast IJP (sensitive to MRS2179 10μM) followed by a sustained IJP (sensitive to L-NNA 1mM) was recorded. In the muscle bath, the spontaneous activity was abolished by NaNP 10μM (n=5) (more than 10 min), in contrast ATP (1mM) or ADPßS (10μM) transiently inhibited the motility but spontaneous contractions partially recovered with time. EFS at 2 Hz during 2 minutes caused a cessation of the spontaneous motility. The same result was obtained in the presence of MRS2179 10μM. A transient relaxation was obtained when tissue was incubated with L-NNA. We conclude 1- Single pulses elicit ATP release from enteric motor neurons that cause a fast IJP and a sharp relaxation that is difficult to maintain over time, 2- Nitric oxide is released at frequencies higher than 2Hz probably causing repetitive action potentials in neurons that cause a sustained hiperpolarization leading sustained relaxation. These differences

might be responsible of complementary mechanisms being phasic (ATP) and tonic (NO) relaxations.
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Interaction of c-terminus of Cav1.2 with c-src kinase via proline-rich region

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We have previously shown that colonic inflammation in the mouse results in significant attenuation of the calcium currents which may involve altered regulation by the non-receptor tyrosine kinase, c-src kinase (Kang et al., 2004). The aim of the present study was to determine if this occurs as a result of altered interaction of c-src kinase with the calcium channel, Cav1.2b, during inflammation and to define the binding site(s) on the channel. Glutathione-S-transferase (GST) fusion proteins were constructed for the C-terminus of the Cav1.2 (amino acids 1865-2172) (GST-CT2) and C-terminus lacking the proline-rich domain (amino acids 1980-1998), denoted as GST-ΔPRD. Src SH2 (amino acids 150-247) and SH3 (83-144) domain fusion proteins were constructed from mouse c-src kinase. Colonic inflammation was induced by intracolonic instillation of TNBS (2.5%) in Balb/C mice. Pull-down of GST-CT2 and GST-ΔPRD with purified c-src followed by immunoblot with anti c-src antibody showed a marked decrease in the interaction of the C-terminus of Cav1.2 with src kinase when the proline-rich domain was deleted. In order to determine if the interaction of c-src from colonic smooth muscle with the C-terminus of Cav1.2 was altered with inflammation, a pull-down assay was performed with GST-CT2 and ΔPRD followed by immunoblot with anti-c-src antibody using smooth muscle cell lysates from control and inflamed tissue samples. The interaction of smooth muscle src with CT2 was not affected by inflammation while that to ΔPRD was significantly reduced. To determine if the PRD domain is necessary for the interaction with c-src kinase, overlay assays of purified CT2 and ΔPRD were performed with GST-SH3 and GST-SH2. Immunoblot with anti-GST antibody showed that CT2 bound to the SH3 domain of c-src but not to SH2. Deletion of the PRD domain prevented binding to both SH3 and SH2 fusion proteins. These data suggest that c-src kinase interacts with the proline-rich region of the C-terminal segment of Cav1.2 via its SH3 domain. This interaction does not appear to be altered during colonic inflammation and indicate that decreased calcium regulation by src kinase may be due to altered phosphorylation.

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Effects of short-chain fatty acids on large intestinal motility and their mode of detection

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Short-chain fatty acids (SCFAs), which are bacterial fermentation products in the large intestine, have been reported to induce physiological responses such as smooth muscle contraction and fluid secretion. Three major SCFAs, containing 2 – 4 carbons, acetate (C2), propionate (C3) and butyrate (C4), each had different effects on the isolated rat distal colon. Propionate induced a phasic contraction and an increase in the frequency of spontaneous contractions. Butyrate also induced a phasic contraction, but not an increase in the frequency of spontaneous contractions. On the other hand, acetate did not induce phasic contractions, but it decreased the frequency of spontaneous contractions. The propionate-induced phasic contraction had two components, rapid, spike-like, contractions and an underlying increase in tone. The former was TTX-sensitive, but the latter was piroxicam-sensitive and TTX-insensitive. The acetate-induced inhibitory effect was also TTX-sensitive. From these results, we have hypothesized that the luminal SCFAs stimulate receptors on the apical membranes of enteroendocrine cells that release mediators from their basolateral aspects to activate sensory neurons in the enteric nervous system (ENS), or that the SCFAs cross the epithelium and directly stimulate sensory nerve terminals and/or some lamina proprial cells. In 2003, the orphan G protein-coupled receptors, GPR41 and GPR43, were reported to be SCFA receptors. We produced antibodies, using synthesized peptides, specific for these receptors, and investigated their distributions in the intestine by RT-PCR, Western blotting and immunohistochemistry. We found that GPR43 is expressed by peptide YY (PYY) enteroendocrine cells and 5-HT-containing mucosal mast cells in the rat intestine. PYY is known to be a hormone that mediates the so-called ileal brake induced by nutrients including SCFAs. It has also been reported that SCFAs stimulate the release of 5-HT in the intestine. Therefore, the localization that we determined is consistent with physiological data previously reported.

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Role of VIP and Substance P in NANC innervation in the longitudinal smooth muscle of the rat jejunum. Influence of extrinsic denervation

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Extrinsic denervation of the transplanted small bowel could play a substantial role in enteric motor dysfunction of the transplanted gut. **Aim:** To determine effects of extrinsic denervation on motor function mediated by the nonadrenergic, noncholinergic (NANC) neurotransmitters Substance P (Sub P) and Vasoactive intestinal polypeptide (VIP). **Methods:** Jejunal longitudinal muscle strips were obtained from 6 groups of rats (n≥6/group): NC (naive controls); SC (sham operated rats); 1 and 8 weeks after jejunal and ileal intestinal transaction/anastomosis to disrupt enteric myoneuronal continuity (TA1 and TA8), and after syngenic jejunoileal transplantation (Tx1 and Tx8). We studied the effect of exogenous Sub P and VIP (10^{-8}; 10^{-7}; 10^{-6}M) on spontaneous contractile activity under NANC-conditions and evaluated antagonistic properties of [D-Pro^2,D-Trp^{7,9}]-Sub P (Sub P antagonist) and [D-p-Cl-Phe^2,Leu^{17}]-VIP (VIP antagonist) with and without NO-synthase inhibitor L-N^G-nitro arginine (L-NNA). Data are Mean±SEM in [%] of baseline contractile activity. **Results:** Spontaneous contractile activity did not differ between groups. Sub P dose-dependently increased contractile activity in all groups, greater in NC compared to Tx8 (355±40% vs. 177±16%; p<0.01). Response to Sub P (10^{-6}M) was less in Tx8 compared with Tx1 (177±16% vs. 306±17%; p=0.001). [D-Pro^2,D-Trp^{7,9}]-Sub P stimulated contractile activity the NC, Tx1, and Tx8 (130±3%, 128±3%, and 132±5%, respectively; p<0.01) but did not block the effect of 10^{-7}M Sub P. VIP dose-dependently decreased contractile activity in all groups 1 week postoperatively (SC: 63±8%; TA1: 57±4%; Tx1: 61±8%); effect of VIP was greater in Tx1 vs. Tx8 (61±8% vs. 93±7%; p<0.01). L-NNA reduced the inhibitory effect of VIP in all groups where VIP had an effect, especially in TA1 (57±4% vs. 83±6% (with L-NNA); p=0.001). In muscle strips precontracted with 10^{-7}M Sub P, VIP (10^{-6}M) reduced contractile activity in all groups (13±2% to 21±5%; p<0.01). The percentage inhibition was greater than the inhibition of spontaneous contractile activity (p<0.01). In precontracted muscles, [D-p-Cl-Phe^6,Leu^{17}]-VIP reversed the inhibition of VIP in SC, TA1, TA8 and Tx8 (71±5% to 84±6%; p<0.005); without precontraction [D-p-Cl-Phe^6,Leu^{17}]-VIP had no effect. **Conclusions:** Chronic extrinsic denervation causes a decrease in contractile response to Sub P in rat jejunal longitudinal muscle. Abdominal operations increase VIP sensitivity, which is reversible after 8 weeks. Effect of VIP seems mediated in part by NO. Precontraction with Sub P increases inhibitory effects of VIP.

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Presence of functional ghrelin receptors on neurons and satellite glial cells in rat dorsal root ganglia

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The peptide ghrelin provides input to the brain on the nutrient status of the gut, both by a hormonal pathway and by activation of vagal afferent nerves. We investigated the ability of ghrelin to activate the spinal visceral afferent pathway involved in sensory reception. The cytosolic Ca^{2+}concentration [Ca^{2+}] of neurons and satellite glial cells (SGC) was recorded (20 ^{0}C) in freshly isolated dorsal root ganglia (D2-D14; T8-T13) loaded with Fluo-4-AM (10^{-6}M; 40 min) on a CLSM (one image/s). Ganglionic vital cells were recognized by their [Ca^{2+}] response to a 10 s perfusion of 70 mM KCl. About 55% of the neurons (52/95; 18 ganglia) responded to GH-releasing peptide (GHRP-6; 10^{-6}M; application 30-60 s) with a fast (mean lag time 11 s) and transient rise in [Ca^{2+}]. The amplitude of the responses was independent on the concentration of GHRP-6 ($10^{-13}$to 10^{-5}M) and was on average 1.16 ± 0.15 RF (Relative Fluorescence; mean ± SD), being 43% of the KCl response. Almost all (91%) SGC responded to GHRP-6, with a lag time of 11 s and amplitude equal to (103 ± 59 %; n=98) the response to KCl (1.62 ± 0.38 RF). Sigmoid dose-response relations ($10^{-14}$to 10^{-5}M), based on the % of responding cells, revealed an EC_{50} of 10^{-11}M for both neurons and SGC. Interestingly, this EC_{50} for the peripherally located ganglion is comparable to previously reported effects of ghrelin on spike activity and [Ca^{2+}] of the hypothalamic arcuate nucleus neurons. Application of ghrelin (rat) did evoke responses in the neurons and SGC comparable to those to GHRP-6 (EC_{50} 10^{-11}M). (Des-octanoyl)-ghrelin (human; range $10^{-10}$to 10^{-7}M) did not evoke responses. The presence of the ghrelin receptor (GHS-R1a) in dorsal root ganglion neurons and SGC was demonstrated at the mRNA level (*in situ* hybridization) and at the protein level (immunocytochemistry, using double-labeling with CGRP to identify viscerally projecting neurons). In conclusion, these results demonstrate the presence of functional ghrelin receptors on visceral spinal neurons and SGC. The results further indicate that ghrelin, in concentrations comparable to those reported in plasma (≥ 10^{-10}M), has an excitatory effect on both ganglionic cell types. This finding supports the notion of a chemosensory role of the SGC and indicates a modulating role of ghrelin in visceral spinal signaling.

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Inhibitory purinergic neurotransmission in human lower esophageal sphincter

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We found in human lower esophageal sphincter (LES) that NO is the main inhibitory neurotransmitter released by inhibitory enteric motor neurons (EMN) and a minor role for an apamin-sensitive neurotransmitter, most probably ATP. Actions of ATP in the gastrointestinal tract can be mediated through metabotropic P2Y receptors and P2X receptors. **Aims:** To characterize the purinergic receptors mediating the human LES relaxation following stimulation of inhibitory EMN. **Methods:** Circular LES muscle strips (3x10 mm) containing the myenteric plexus from 8 patients undergoing surgery for esophageal cancer were studied *in vitro* in the organ bath. **Experimental design:** Pharmacologic effect of specific $P2Y_1$ receptor agonists (ADPβS y 2-MeSATP) and the selective P2X receptor agonist (α,β-meATP) b) Stimulation of EMN with electrical field stimulation (EFS, 0.4 ms, 0.3-20 Hz, 26 V) or trough nicotinic receptors (nAChRs) by nicotine (100μM); and characterization of the LES relaxation by inhibition of NO synthesis with L-NNA (1mM) and with the specific $P2Y_1$ receptor antagonist MRS 2179. **Results:** In the presence of tetrodotoxin (TTX 1μM) the $P2Y_1$ receptor agonists ADPβS (10-100μM) and 2-MeSATP (10-100μM) caused a biphasic response with an initial fast LES relaxation and a sustained contraction. The P2X agonist α,β-meATP (10-100μM) induced only a sustained LES contraction. b) EFS induced a frequency-dependent relaxation of LES strips blocked by TTX 1μM. Inhibition of NO synthesis by L-NNA 1mM significantly reduced LES relaxation induced by EFS (20Hz: -50.4 ± 8.8 % of basal relaxation), and the sequential addition of MRS 2179 (10 μM) further decreased the non-nitrergic electrical induced relaxation (20 Hz: −24.30 ± 8.9 %, p<0.05). Stimulation of inhibitory MNs by Nicotine also induced a LES relaxation partially antagonized with L-NNA (−52.54 ± 6.85 %, p<0.05). The non nitrergic relaxation induced by nicotine is further reduced with the specific $P2Y_1$ receptor antagonist MRS2179 (−25.50 ± 14.53 %, p<0.05). **Conclusions:** Human LES relaxation induced by stimulation of inhibitory motor neurons of the myenteric plexus by EFS or trough nAChRs is mainly mediated by release of NO and a minor contribution of a purine acting through purinergic $P2Y_1$ receptors. P2X receptors might mediate excitatory responses in human LES. (FIS PI/02662, SGR 2005-00255).

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Selective stimulation of excitatory and inhibitory motor neurons in porcine lower esophageal sphincter

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We found that stimulation of inhibitory motor neurons (MN) in porcine lower esophageal sphincter (LES) releases NO and ATP (acting at $P2Y_1$ receptors through apamin-sensitive mechanisms), and stimulation of excitatory enteric MN releases acetylcholine (ACh). Synapses to EMN can be mediated by nicotinic receptors (nAChRs), ATP acting on purine P2X receptors and serotonin (5-HT) acting on 5-HT3 receptors**.**

Aim: To assess the effects of selective stimulation of inhibitory and excitatory MN through nAChRs, 5-HT3 and P2X receptors in porcine LES. **Methods:** Circular transmural LES muscle strips (clasp region, 3x10 mm) from 20 adult pigs were studied *in vitro* in the organ bath. Design: a) Study of excitatory and inhibitory responses in a basal Kreb's solution, in a non-adrenergic, non-nitrergic, non-purinergic (NANNNP) Kreb's solution (propranolol 1μM, phentolamine 1μM, L-NAME 1mM, and apamin 1μM) for selective study of excitatory EMN; and in a non-adrenergic, non-cholinergic (NANC) solution (propranolol 1μM, phentolamine 1μM and atropine 1μM) for the selective study of inhibitory EMN. b) Stimulation of nAChRs by nicotine (1-100μM), of P2X receptors by α,β-Methylene ATP (α,β-meATP 1-100μM) and of serotoninergic receptors by 5-hydroxytriptamine (5-HT, 0.001-100μM). **Results:** In basal Krebs solution Nicotine (1-300μM) caused an intense concentration-dependent LES relaxation that was significantly reduced by L-NAME (1mM) and the neurotoxin TTX (1 μM). 5-HT (0.001-100μM) evoked a concentration dependent contraction that was significantly reduced by TTX and atropine (1μM). The specific P2X receptor agonist α,β-meATP (1-100μM) caused a sustained contraction in 60% of LES strips while it evoked a long lasting relaxation in 40% of strips. 2) In the NANC Krebs solution, 5-HT still evoked a contraction and α,β-meATP induced a consistent LES relaxation. 3) In NANNNP conditions α,β-meATP always caused a sustained contraction that was antagonized by TTX, and Nicotine did not induce any effect. **Conclusions:** Inhibitory EMN of porcine LES can be predominantly stimulated through nAChRs, and excitatory EMN mainly stimulated by 5-HT receptors. P2X receptor agonists might stimulate both inhibitory and excitatory MNs. Selective stimulation of inhibitory or excitatory EMN in the myenteric plexus might be a potential tool for treatment of human LES diseases. (FIS PI/02662, SGR 2005-00255).

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Neuroprotective/trophic effects of 5-HT_4 receptor stimulation on enteric neurons of mice

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The number of neurons in the enteric nervous system (ENS) changes as a function of age. In the mouse, new neurons are added to the ENS through ~P21. The number of enteric neurons then stabilizes, but

ultimately falls in later life. In most mammals about 40-60% of neurons are ultimately lost. The functional consequences of the age-related decrease in enteric neurons are not completely clear, but it may contribute to the high incidence of dysmotility in the aged. The 5-HT_4 agonist, tegaserod, is effective in the treatments both of chronic constipation and irritable bowel syndrome-constipation; moreover, colonic transit slows in aged mice and does so even more in transgenic mice that lack 5-HT_4 receptors (KO). Because the numbers of neurons are also reduced in both plexuses of KO mice, however, the KO-associated defect in colonic transit could be due to a loss of the 5-HT_4 effects on neurotransmission and/or to the loss of neurons. We therefore tested the hypothesis that 5-HT_4 stimulation is neuroprotective or trophic. Numbers of myenteric neurons in the colon were compared between KO and wild-type (WT) littermates at 1, 2.5, 4, 5, and 12 months of age. Neural numbers were not significantly different at 1 month, but at each of the later ages, there were significantly fewer neurons in the KO colon (the mean WT/KO ratio was 1.6 ± 0.1; $p < 0.05$). Neuronal size and the proportion of myenteric nNOS-immunoreactive neurons were also decreased in KO mice at 12 months, but not at younger ages. Feret's diameter was 21.5 ± 0.3 μm in KO and 23.2 ± 0.4 μm in WT mice ($n = 100$, $p < 0.01$); the nNOS/Hu ratio was 31.4 ± 2.1% in KO animals and 39.2 ± 1.8% in WT ($n = 15$, $p < 0.01$). To study trophism and/or neuroprotection more directly, 5-HT_4 agonists (tegaserod and RS67506) and 5-HT_4 antagonist (GR113808) were applied to enteric neurons developing *in vitro* from immunoselected neural crest-derived precursors. Both tegaserod and GR113808 concentration-dependently increased neuronal numbers and length of neurites; these effects were blocked by GR113808, which exerted no effects of its own. Tegaserod and RS67506 decreased apoptosis, assessed by the TUNEL method; these decreases were blocked by GR113808. These observations suggest that 5-HT_4 receptor stimulation is neuroprotective and trophic for enteric neurons. Whether or not 5-HT_4 stimulation can prevent the age-related decline in neuronal numbers remains to be determined. Supported by NS12969, NS15547 and Novartis.

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Effects of cannabinoids on NANC neural transmission in mouse colon

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It is well accepted that endogenous cannabinoids and CB1 receptors are involved in the regulation of smooth muscle contractility and intestinal motility, through a mechanism mainly related to the reduction of acetylcholine release from cholinergic nerve endings. Therefore, the present study sought to investigate the effects of cannabinoid drugs on non-adrenergic non-cholinergic (NANC) contractile and relaxant responses to the circular muscle of mouse proximal colon elicited by electrical field stimulation (EFS).

EFS (trains of 5 s; 0.5 ms, supramaximal voltage, 2-32 Hz) in the presence of atropine and guanethidine (NANC conditions) evoked a tetrodotoxin-sensitive response, consisting in a small relaxation followed by contraction. The EFS-evoked contraction enhanced in amplitude as the stimulation frequency was increased. Moreover, it was significantly reduced by SR48968, NK2 receptor antagonist, suggesting that it mainly was due to release of neurokinin A.

The cannabinoid receptor agonist WIN 55,212-2 (1 – 100 nM), the putative endogenous ligand anandamide (10 -100 μM), the selective CB1 receptor agonist ACEA (0.01 – 1 μM), but not the selective CB2 receptor agonist JWH-015 (0.1 μM), produced a concentration-dependent reduction of the NANC contractile responses, without affecting the NANC relaxation. WIN 55,212-2, anandamide and ACEA did not modify the contractions produced by exogenous [(-Ala^8]-NKA(4-10), agonist of NK2 receptors. The selective antagonist of CB1 receptors SR141716A, *per se* failed to affect the EFS-evoked responses, but antagonized the inhibitory effects of WIN 55,212-2, anandamide and ACEA on NANC contractile responses.

The present results suggest that activation of prejunctional cannabinoid CB1 receptors produces inhibition of NANC contractile responses in mouse colonic circular muscle. However, endogenous ligands do not seem tonically modulate the NANC transmission in mouse colonic circular muscle.

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Syntaxin1A regulatory proteins are not present in esophageal smooth muscle

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Background: Syntaxin 1A (Syn1A), a t-SNARE protein is essential in membrane fusion, and is in dynamic conformational equilibrium, between closed and open states. Its conformational transition from the closed to the open state is a key regulatory element in exocytotic membrane fusion and ion channel modulation in secretory cells. In secretory cells, the regulatory proteins, munc-18 and munc-13 facilitate the rearrangement in Syn1A structure. Munc-18 stabilizes a closed Syn1A conformation by binding to the whole Syn1A protein, while munc-13 binding to the N-terminal domain of Syn1A displaces the munc-18, potentially facilitating the transition of Syn1A to an open conformation. As opposed to secretory cells where the open state Syn1A has a greater inhibitory effect on ion channel modulation, in smooth muscle cells only the wild type, or closed form of Syn1A, regulates the ion channels. This finding suggests that munc-18 and/or 13 actions are unnecessary in some nonsecretory cells such as smooth muscles. **Hypothesis:** In nonsecretory cells such as smooth muscle, munc-18 and 13 are absent. **Methods:** We investigated the cellular localization of mammalian munc-18 isoforms at the protein level in esophageal smooth muscle (ESM) using sub-cellular fractionation combined with western blotting. Furthermore, *in vitro* GST-fusion protein pull-down assays were used to detect any diminutive amount of endogenous munc proteins in ESM extracts that might not be detected on a western blot. **Results:** Incubation of rat brain homogenate with antibodies raised against several mammalian munc-18 isoforms (Munc-18a, b, and c) revealed strong bands. However, in subcellular fractions of smooth muscle cells, there were no corresponding munc protein bands in either the cell cytosol or membrane fractions. Munc-13 was also absent in the ESM. Furthermore, GST-Syn1A which has a great affinity for munc proteins was unable to pull down any munc-18a or b or, munc-13 from ESM, confirming the absence of these proteins in the smooth muscle. With rat brain lysate, both wild type and open form of GST-Syn1A bound munc-18a and b as well as munc-13. **Conclusions:** Munc-18 and 13 are not present in the nonsecretory smooth muscle cells of the esophagus where only the wild type Syn1A has an inhibitory effect on ion channels. Therefore, it appears that a change to an open conformation Syn1A, normally facilitated by munc-18/13, is not required. The absence of munc proteins is consistent with the concept that Syn1A regulation of ion channels in nonsecretory cells may be different than in secretory cells.

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PGE2 increases phospholamban phosphorylation and CaM kinase II activity in murine proximal colon smooth muscles

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The prostaglandin PGE2 is implicated in the digestive tract dysmotility associated with Crohn's disease, ulcerative colitis, and postoperative ileus. PGE2 reduces the contractile activity of colonic circular smooth muscles. The EP2 and EP4 PGE2 receptors are coupled to adenylyl cyclase by Gαs, resulting in elevated cAMP levels and PKA activation. Similar to cardiac muscle, it has been shown that cAMP-evoked smooth muscle relaxation involves lowering $[Ca^{2+}]_i$ and elevating SR Ca^{2+} levels by activation of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). SERCA activity is regulated by the SR membrane protein phospholamban (PLB). Phosphorylation of PLB by PKA or PKG at Ser16, or by CaM kinase II at Thr17 removes its inhibitory effects on SERCA, thereby enhancing SERCA activity. Ca^{2+}

mobilization is altered in circular smooth muscle cells in animal models of inflamed colon. The elevation of cAMP and activation of PKA by PGE2 suggest that PGE2 is a likely candidate to increase PLB phosphorylation and alter proximal colon smooth muscle Ca^{2+} handling dynamics and CaM kinase II activity. These findings led us to investigate the effects of PGE2 on PLB phosphorylation and CaM kinase II activity in murine proximal colon smooth muscles. PGE2 dose-dependently (1 nM-5 nM) decreased the amplitudes of proximal colon circular smooth muscle spontaneous phasic contractions. The PGE2-evoked relaxation of proximal colon circular smooth muscles was inhibited by the Gαs inhibitor NF449 (15 μM), the adenylyl cyclase inhibitor SQ22536 (5 μM), and the PKA inhibitor H-89 (0.5 μM). In addition, 2-APB (25 μM) inhibited the PGE2-evoked proximal colon circular smooth muscle relaxation. PGE2 also increased CaM kinase II activity in a dose-dependent manner and 2-APB inhibited CaM kinase II activation by PGE2. PLB Ser16 and Thr17 phosphorylation were increased in PGE2-treated proximal colon smooth muscle tissues. Both PLB Ser16 and Thr17 phosphorylation were inhibited by H-89. However, only PLBThr17 phosphorylation was inhibited by 2-APB or KN-93. These findings indicate that CaM kinase II and PLB are downstream targets of PGE2 in proximal colon smooth muscles, and suggest that the PGE2-evoked relaxation of murine proximal colon circular smooth muscles via the Gαs/adenylyl cyclase/ PKA pathway involves altered SR Ca^{2+} handling dynamics.

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Role of c-src kinase in inflammation-induced altered contractility of murine colon

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Calcium currents and smooth muscle contraction are significantly attenuated during colonic inflammation. Our laboratory has previously demonstrated altered regulation of the calcium currents by the non-receptor tyrosine kinase, c-src kinase, in colonic smooth muscle in the dextran sulphate model of colitis. The aim of the present study was to determine the role of c-src kinase in altered contractility of colonic smooth muscle during TNBS-induced colitis. Colonic inflammation was induced by intracolonic instillation of TNBS (2.5% in 50% ethanol) in male Balb/C mice. Isometric tension recordings of distal colonic smooth muscle were performed from both control and inflamed (3 day post-TNBS) mice. In order to determine the effects of c-src on calcium influx in tissues, cumulative $CaCl_2$ dose-dependent contractions were elicited in colonic strips depolarized by high potassium (80 mM) in the presence and absence of the c-src kinase inhibitor, PP2 (10 μM). The maximal contractile response to calcium was significantly less in inflamed tissues (38.4 ± 7.6 %) compared to controls ($p>0.05$; n=8). The EC_{50} for $CaCl_2$ was 1.2×10^{-4} M in controls and 2.4×10^{-4}M in inflamed tissues indicative of reduced calcium sensitivity in inflamed tissues. In the presence of PP2 (10 uM), the pD_2 was shifted from 3.88 to 2.44 (n=4) in controls while it remained largely unaffected in inflamed tissues (pD_2 :3.66 vs 3.43) suggesting that c-src kinase was less effective in modulating depolarization-induced calcium influx in inflamed colon. In whole cell voltage clamp studies of single smooth muscle cells, PP2 (10 uM) attenuated calcium currents from −10.3 ± 1.3 pA/pF to −5.2 ±0.4 pA/pF (n=10) in control and from −5 ± 1.1 pA/pF to −3.2 ±0.5 pA/pF (n= 8) in inflamed cells, respectively. These data suggest that c-src kinase regulation of smooth muscle contractility is altered following inflammation and corroborate the electrophysiological data from single smooth muscle cells.

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Chronic administration of corticotropin-releasing hormone in rats causes intestinal epithelial dysfunction by activating mucosal mast cells

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Background: Our previous studies reported that psychological stress in rodents enhances intestinal epithelial ion secretion and increases permeability for macromolecules and bacteria. Corticotropin-releasing hormone (CRH) is a key stress mediator and exerts its effects through CRH receptor ligation on effector cells, possibly mast cells. We found that a CRH antagonist injected IP before stress inhibited the pathophysiology. However, the exact pathway involved in the mechanism of the stress-induced intestinal dysfunction remains unclear. **Aims:** To investigate changes in colonic function and define the role of mast cells in gut pathophysiology induced by chronic CRH administration in rodents. **Methods:** Wild type rats (+/+) and their mast cell-deficient (Ws/Ws) littermates were surgically implanted (sc) with osmotic minipumps containing CRH (50μg /kg/day). Minipumps with normal saline were implanted in control rats. After 10 days, intestinal segments were excised and mounted in Ussing chambers. Colonic ion secretion was measured by short-circuit current (Isc) and macromolecular permeability by the flux of horseradish peroxidise (HRP). In vitro studies of epithelial barrier function were carried out in T84 epithelial cell monolayers co-cultured with CRH-stimulated mast cells (HMC-1 or RBL cells); barrier function parameters were assessed by measuring the transepithelial resistance (TER) and HRP flux. CRH receptor expression in mast cells was determined by RT-PCR and Western blots. Corticosterone levels in serum were determined by RIA. **Results:** Both +/+ and Ws/Ws groups of rats treated with CRH showed increased corticosterone levels in serum compared to saline controls. However, only the +/+ rats treated with CRH showed significantly enhanced Isc and HRP flux compared with +/+ controls and Ws/Ws rats. Addition of CRH to T84 monolayers co-cultured with mast cells substantially decreased the epithelial TER and increased HRP flux compared with controls. These changes were not present when mast cells were excluded. Expression of CRH receptors was detected in mast cells at mRNA and protein levels. **Conclusions:** Chronic administration of CRH mimics the effect of chronic stress in compromising intestinal barrier function. CRH activates mast cells with subsequent release of mast-cell-derived mediators that have direct effects on the epithelium to increase ion secretion and impair barrier function. Mast cell stabilization may improve intestinal pathophysiology in gut disorders provoked by stress.

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Cellular distribution of P2 receptor subtypes in the rat distal colon

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Previous studies have indicated that more than one pre- and postsynaptic P2 receptor is involved in the relaxant effect of ATP on the rat distal colon circular muscle. P2 receptors are subdivided into two families: ionotropic P2X ion channels and metabotropic P2Y receptors. To date, seven P2X subunits ($P2X_{1-7}$) and eight P2Y receptors ($P2Y_{1,2,4,6,11-14}$) are accepted as clearly defined nucleotide receptors. Based on previous data concerning the role of P2 receptors in colon, this study focussed on the distribution of the P2 receptor subtypes to reveal which of these is involved in the relaxant effect of ATP on rat colon circular muscle. $P2X_1$ and $P2X_4$ immunoreactivity (IR) were detected in resident macrophages. $P2Y_1$, $P2Y_6$, and a weak $P2X_1$ IR were found in enteric smooth muscle cells. $P2X_1$ IR was also observed in enteric glial cells (EGCs) of myenteric ganglia. EGCs of both plexuses expressed $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2X_6$ and $P2X_7$ IR. Nearly all enteric neurons were immunostained for $P2X_2$. $P2Y_2$ was demonstrated in 50±4% of the myenteric neurons. These neurons had a typical Dogiel type I morphology, and 23±3% of them were nitrergic neurons. In the myenteric plexus, 26±4% of the neurons expressed $P2Y_{12}$, while in the submucous plexus 67±6% expressed $P2Y_{12}$. A part of the $P2Y_{12}$-immunoreactive neurons were large-sized and smooth-contoured, resembling a Dogiel type II morphology, while the remaining part had a Dogiel type I morphology. $P2Y_{12}$-expressing myenteric neurons did not co-express nNOS, while colocalisation of $P2Y_{12}$ and nNOS was observed in 20±9% of the submucous $P2Y_{12}$-expressing neurons. $P2Y_{14}$ IR was detected in 34±6% of the submucous neurons, of which 38±5% co-expressed nNOS. Furthermore, individual $P2X_3$-immunoreactive

nerve fibres scattered throughout the myenteric plexus were observed. $P2Y_{13}$ and $P2X_5$ were, just like $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, $P2X_2$, $P2X_3$ and $P2X_6$, demonstrated in colonic epithelial cells. Each of the P2 receptors was expressed in specific cell types in the rat distal colon. The present morphological results suggest a major involvement of muscular $P2Y_1$ and neuronal $P2X_2$ receptors in the relaxant effect of ATP on the rat distal colon circular muscle. A putative role for the $P2Y_2$ receptors expressed in nitrergic neurons and for the muscular $P2X_1$ receptors as well as for the P2 receptors expressed on the EGCs cannot be excluded. *This study was supported by the IUAP project P5/20.*

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Changes in the expression pattern of somatostatin receptor subtypes during intestinal inflammation in the mouse

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Although previous studies have shown the involvement of somatostatin-mediated pathways in the gastrointestinal tract (GIT) under (patho-)physiological conditions, little information is available on somatostatin receptors (SSTRs) mediating these effects. In the rat GIT, contradictory results regarding SSTR distribution patterns are reported. In the murine GIT, although the presence of other SSTRs has been suggested, only SSTR2A has been shown in both enteric plexuses. This study aimed to investigate the expression of all five SSTRs in the non-inflamed and *Schistosoma mansoni*-infected murine ileum using immunohistochemistry, RT-PCR and Real Time PCR. In the non-inflamed ileum, SSTR1 immunoreactivity (IR) was found in enterocytes, in some cells of the crypts of Lieberkühn, and in nerve fibers in both enteric plexuses and at the base of the crypts. SSTR2A was detected in myenteric neurons, submucosal glial cells and in nerve fibers at the base of the crypts. SSTR4 IR was present in submucosal and myenteric nerve fibers. Neither SSTR3 nor SSTR5 was detected. The acutely *S. mansoni*-infected ileum is characterized by a mucosal mast cells (MMC) recruitment and neuronal sprouting in the lamina propria. Both MMC and nerve fibers in the lamina propria expressed SSTR1 and SSTR3. SSTR4 IR nerve fibers were detected in granulomas and the lamina propria. These results were confirmed by RT-PCR and quantitative Real Time PCR analysis, indicating a significant increased mRNA level of SSTR1 and SSTR3 and a slightly increased mRNA level of SSTR2 and SSTR4 in the inflamed ileum. This study revealed that SSTRs are expressed in specific cell types in the murine ileum. The expression of SSTR1 and SSTR3 on MMC and the increased density of somatostatin-expressing nerve fibers in the lamina propria during intestinal schistosomiasis, support the hypothesis that somatostatin is involved in the physiological control of MMC and has a modulatory effect during intestinal inflammation. Finally, evidence is provided that mainly SSTR1 and SSTR3 are involved in the inflammatory effects of somatostatin during intestinal schistosomiasis. *This study was supported by the IUAP project P5/20, FWO grant G.0377.04 and an IWT fellowship (SB51449) to JVOdb.*

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Sensitization of P2X receptors contributes to the visceral hyperalgesia in a rat model of irritable bowel syndrome

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Irritable bowel syndrome (IBS) is a common gastrointestinal disorder and characterized by abdominal pain and bloating in association with altered bowel movements. However, its pathogenesis and underlying molecular mechanisms of visceral hyperalgesia remain elusive. Recent studies of somatic and other visceral pain models suggest a role for purinergic signaling mediated by the P2X receptor family. In this study, we used a rat model of IBS-like visceral hyperalgesia to examine a role for P2X signaling in the pathogenesis of the same. "IBS" was induced by colonic injection of acetic acid (AA) in 10 day old rat. Dorsal root ganglion (DRG) neurons innervating the colon were labeled by injection of DiI fluorescence into the colon wall one week before electrophysiological studies. Patch-clamp recordings were performed from acutely dissociated DRG neurons 8 weeks after colonic infusion of AA or vehicle. Rapid application of ATP (20 μM) depolarized membrane potentials in neurons from both control and AA-treated rats. ATP evoked a larger membrane depolarization in AA-treated rats than in control rats. Under voltage-clamped conditions, ATP induced fast inactivated currents in these neurons. These currents were sensitive to TNP-ATP (100 nM), a selective antagonist of P2X receptor. The most noticeable change was a 2-fold-increase in the peak ATP responses in AA-treated rats. Further, P2X3 receptor protein expression was significantly increased in colon specific DRGs 8 weeks after neonatal AA treatment. In addition, systemic administration of TNP-ATP (50 mmol/kg, i.p.) markedly reduced the pain behavioral response to colon distension in rats with hypersensitivity. These data suggest that the large enhancement of ATP receptor expression may contribute to the pain sensitivity of colon sensory, thus identifying a specific a neurobiological target for the treatment of chronic visceral hyperalgesia.

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ATP contributes to excitatory enteric neurotransmission in the longitudinal muscle of mouse distal colon

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In mouse gastrointestinal tract, adenosine 5'-triphospate (ATP), acting via P2 purinoceptors, has been reported to be involved in inhibitory as well in excitatory enteric neurotransmission. The aim of the present study was to investigate, using *in vitro* mechanical recordings, the role of ATP on enteric neurotransmission in longitudinal muscle of mouse distal colon. Activation of intrinsic neurons by electrical field stimulation induced a transient nitrergic relaxation, followed by a contraction reduced by atropine, by PPADS or suramin, non selective P2 purinoceptor antagonists, and by P2Y receptor desensitization with ADPβS, selective P2Y receptor agonist. Desensitization of P2X receptors with α,β-methylene ATP (α,β-meATP) had not significant effects on the evoked responses. Indeed, pre-treatment with MRS 2179, specific P2Y1 antagonist, caused an increase in the contractile response, without affect the evoked relaxation. Non cumulative addition of ATP (??μ??- 1 mM) or ADPβS (0.3 - 100 μM) caused a concentration-dependent contraction, being ADPβS more potent than ATP. Indeed, 2-MeSATP (0.3 - 30 μM), P2Y1 receptor agonist induced a small relaxation. Contractile responses to ATP and ADPβS were partially reduced by atropine and deeply antagonized by PPADS, suramin or by P2Y receptor desensitization with ADPβS (100 μM for 20 min). Indeed, purinergic contractions were increased in the presence of MRS 2179 (1 μM), TTX (1 μM) and L-NAME (100 μM), an NO synthase inhibitor. These results suggest, in the longitudinal smooth muscle of mouse distal colon, an involvement of ATP in excitatory neurotransmission via activation of ADPβS-sensitive P2Y purinoceptors, located on the smooth muscle and on the cholinergic neurons. P2Y1 purinergic receptors appear to be located on nitrergic inhibitory neurons, regulating the release of NO.

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Motility patterns in the distal bowel associated with luminal acidic pH changes

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Introduction: With the passage from the small intestine into the colon, the intraluminal environment changes. The cecum, compared to the distal ileum, represents a potentially less contractile reservoir where colonic bacteria cause an acidic change in pH. The gut passage

of a non-digestible solid with pH and pressure sensors (SmartPill capsule) may be able to document this transition. **Hypothesis:** A significant pH drop is seen hours after the gastric emptying. We hypothesize that the pH drop recorded by the capsule is due to its ileo-cecal passage and is associated with motility changes. **Aims:** To quantify and compare the motility patterns (pressure and amplitudes of contractions) before and after the pH drop. **Methods:** 104 volunteers (49 males, 55 females) swallowed the SmartPill (SP) after an overnight fast together with a standardized meal and 120 cc water. The rapid pH changes from acidic to alkaline (> 4 and at least 3 unit rise from baseline gastric pH) marked the emptying of the ingested capsule from the stomach into the duodenum. On the capsule's recordings, approximately 5.5 hours after the capsule's gastric emptying, a drop in pH > 1 unit for more than 5 minutes is seen. The frequency and amplitude of contractions were analyzed from 30 min before the beginning of the pH drop to 30 min after. These parameters were then compared by two-sample unequal variance t test. **Results:** The average time from the gastric emptying to the pH drop is 5 hrs 23 min. The frequency of contractions for the 30 min before the pH drop is 3.99 contractions/min (95% CI 3.99±0.014), and for the 30 min after the drop is 2.1 contractions/min (95% CI 2.1±0.01), p<0.0001. The mean amplitude of contractions was not different between the time periods chosen (19.6 mmHg before, 19.4 mmHg after the pH drop, p = 0.8). The motility index for the 30 min before the pH change is 1.54, for the 30 min after is 0.91, p<0.0001. **Conclusion:** The pH drop that appears after the gastric emptying is associated with a highly significant change in motility patterns suggesting that the combined change in pH and motility could mark the transition between the distal ileum and the ceacum. Research funded by SmartPill Corporation.

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Expression of the apical sodium dependent bile acid transporter (ASBT) in a *Trichinella Spiralis* mouse model of post-infective gut dysfunction

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Background/Aim: The observation of disturbed bile acid (BA) function in irritable bowel syndrome (IBS) suggests alterations in BA absorption could contribute to IBS-associated diarrhoea. Bile salt absorption and expression of the ASBT was examined in intestinal tissues from a *Trichinella spiralis* mouse model of post-infectious (PI) gut dysfunction. **Methods:** *T. spiralis* infected mice were sacrificed at 18 and 25 days PI. *Measurement of* ^{3}H-Taurocholic Acid Absorption: The absorption of taurocholic acid (TCA) was determined in everted jejunal and ileal sacs following addition of ^{3}H-labelled TCA to the mucosal fluid. Uptake of label into both the serosal fluid and tissue were used to determined active transport function. *Western blot analysis:* Epithelial cell homogenates were prepared from terminal ileum. Equal amounts of protein were separated by electrophoresis on 10% SDS-polyacrilamide gel and expression determined using a polyclonal antibody against ASBT (Santa Cruz Biotechnology, CA, USA) with a peroxidase-conjugated secondary antibody. The visualization was achieved by enhanced chemiluminiscence technique. *Immunohistochemistry:* Mouse jejunum and ileum were fixed in 4% paraformaldehyde and stained with a polyclonal antibody against ASBT (Santa Cruz Biotechnology, CA, USA) and visualized using FITC. **Results:** Passive 3H-TCA uptake was increased in the jejunum at 18 days PI and recovered at day 25. In contrast active TCA uptake was significantly reduced in the ileum at 18 days PI when compared with controls with a partial restoration at 25 days PI. Western blot analysis showed a protein band of 45 kDa for ASBT in the distal ileum. Inmunohistochemistry studies detected ASBT expression at the apical pole of epithelial cells along the length of the entire villus in the distal ileum. ASBT expression in distal ileum was increased at 18 days PI compared with controls. **Conclusions:** Active bile acid absorption is impaired in a *T. spiralis* model of PI gut dysfunction. However, this does not correlate with decreased expression of ASBT, which is increased PI. The mechanisms underlying impaired bile acid transport remain to be resolved. This study was funded by the Bardhan Research and Education Trust (328452), UK; The Government of Aragon/ ESF (B010/2003) and DGA (CONSI+D) and CAI (CM 7/05), Spain.

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Melatonin antagonizes the effect of LPS in rabbit duodenum

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Background/Aim: The gastrointestinal tract contains high concentrations of melatonin, which is locally produced in enterochromaffin cells. It is known that melatonin can modulate gastrointestinal ion transport processes and motility. To study the intestinal motor disturbances evoked by infectious agents, we used lipopolysaccharide (LPS) of *Escherichia coli*. The aim of the present study was to determine the role of the melatonin in the effect of LPS on the acetylcholine (ACh)-induced contractions in rabbit small intestinal *in vitro*. **Material and Methods:** Pieces of duodenum were cut into small segments (10 mm long and 5 mm wide), suspended in the direction of longitudinal or circular smooth muscle fibres in a thermostatically controlled organ bath containing Krebs solution at 37 ordm;C to reach pH 7.4, continuously gassed with 95% O_2 and 5% CO_2. Each segment was connected to an isometric force transducer with an initial tension of 20 mN. The mechanical activity was amplified and recorded for further analysis using MacLab Systems software. **Results:** LPS (0.3 μg/ml) reduced the contractions induced by ACh (10^{-4} M) in longitudinal and circular smooth muscle of duodenum. However, the amplitude and frequency of spontaneous contractions were not modified by LPS. ACh-induced contractions were not altered by melatonin (0.03; 0.1 and 0.3 mM) in both types of smooth muscle. Melatonin (0.3 mM) antagonized the effects of the LPS on ACh-induced contractions in duodenal smooth muscle. **Conclusion:** Our results show that melatonin reverts the effect of LPS on ACh-induced contractions in rabbit duodenum. These results suggest that melatonin might attenuate the intestinal motor disturbances associated to infectious agents. Supported by DGI AGL2003-03291 and DGA A24/2005 (Spain).

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The involvement of 5-HT$_2$ receptor subtypes in mediating a contraction response to serotonin (5-HT) in *Suncus murinus* intestine

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5-HT$_2$ receptors play a dominant role in mediating the contractile actions of 5-HT in all regions of the intestine of Japanese House Musk shrew *Suncus murinus* (Javid & Naylor, 1999). The aim of the present study was to investigate further the 5-HT$_2$ receptor subtype(s) involved in 5-HT-induced contraction in *Suncus murinus* intestine. Segments (1 cm Length) taken from different regions of the intestine of adult, *Suncus murinus* (32-69 g) of either sex were mounted in 10 ml organ baths containing Krebs' solution (37ºC, 95% O2, 5% CO2). The tissues were allowed to equilibrate for 60 min under 0.5 g tension, and washed every 20 minutes. Using a paired experimental design, non-cumulative concentration-response curves to 5-HT (3.0 nM-0.3 μM) were established in the absence and presence of 1.0 μM of MDL-100907, RS-127445 or SB-242084, 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptor antagonists respectively (Bonhaus, et al., 1999; Kehne et al., 1996; Kennett, et al., 1997) Isometrically recorded tension changes were expressed as the mean±s.e.mean of percentage of KCl-induced contraction (0.12 mM) of n=6 and analysed using ANOVA followed by Bonferroni-Dunnett's t-test. 5-HT (3.0 nM-0.3 μM) induced concentration-dependent contraction in all tissues examined. The application of MDL-100907 significantly (p<0.05) attenuated the contractions induced by 5-HT without affecting the maximum response in segments taken from the

terminal but not the proximal and central regions of the intestine. RS-127445, in tissues taken from the central and terminal but not the proximal regions, attenuated the contraction responses to 5-HT significantly ($p<0.05$) whilst the maximum responses were not affected. SB-242084 attenuated the contraction responses to 5-HT in the proximal and central tissues ($p<0.05$) but not the terminal regions of the intestine, without affecting the maximum response. In conclusion the contractile effects of 5-HT are mediated via 5-HT_{2C} receptors in the proximal intestine. via 5-$HT_{2B/2C}$ receptors in the central intestine and via 5-$HT_{2A/2B}$ receptors in the terminal regions of intestine. The results provide the first evidence for the involvement of 5-HT_{2C} receptors in mediating a contraction response in the intestine. Bonhaus, et al., 1999. Br. J. Pharm., 127, 1075-1082. Javid & Naylor, 1999. Br. J. Pharm., 127, 1867-1875; Kehne et al., 1996. J.Pharm.Exp.Ther., 277, 968-981. Kennett, et al., 1997. Neuropharm., 36, 609-620.

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The effect of reboxetine on electrical field stimulation (EFS)-induced contraction in the rat small intestine

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Fluoxetine at 1.0 or 10 μM has been shown to reduce or abolish the EFS induced contraction responses in tissues taken from all regions of the rat small intestine (Farajian mashhadi et al., 2003). The aim of the present study was to investigate the effect of the selective norepinephrine reuptake inhibitor reboxetine on EFS-induced contractions in different regions of the rat small intestine.

Segments (1 cm Length) taken from different regions of the intestine of male Hooded Lister rats (Bradford strain) weighing 200-300g were mounted in 10 ml organ baths containing Krebs' solution (37 ^{o}C, 95% O2, 5% CO2). The tissues were allowed to equilibrate for 60 min under 0.5 g tension, and washed every 20 minutes. The contractions were recorded isometrically. EFS (0.4, 1.0 and 10.0 Hz, 30.0 V and 0.5 ms pulse width, double pulses with 75.0 ms delay), with a 10.0 min interval of stimulation was delivered by means of two platinum electrodes placed on either sides of the tissue. Using a paired experimental design, the tissues were exposed to EFS for 1.0 min in the absence and presence of reboxetine (1.0-10.0 μM). Tension changes were expressed as the mean±s.e.mean of the percentage of Kcl-induced contraction (0.12 mM) of n=6 and analysed using ANOVA followed by Bonferroni-Dunnett's t-test.

EFS (30.0 V, 0.5 ms width) applied for 1.0 minute induced a relaxation prior to a contractile response at low frequencies (0.4 and 1.0 Hz) and a phasic contraction followed by tonic contraction at a high frequency of 10.0 Hz. Reboxetine at 1.0 μM significantly ($p<0.05$, 0.01) reduced the contraction responses to EFS at different frequencies in segments taken from the jejunum and terminal ileum. At a higher concentration of 10.0 μM, reboxetine attenuated ($p<0.05$, 0.01 and 0.001) the contraction response in the jejunum and ileum at all frequencies, and in the duodenum at 10.0 Hz. Reboxetine at 10.0 μM revealed a relaxation response in all tissues when EFS was applied at 10.0 Hz in all tissues examined ($p<0.01$ and $p<0.001$). The data indicate that noradrenaline is affecting the EFS-induced responses and the role of endogenous noradrenaline in the lower gut might be relevant to the clinical gastrointestinal side effects of reboxetine, i.e. constipation.

Farajian mashhadi, F. et al., 2003. Br. J. Pharmacol., vol 1,047P.

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Slow wave frequency- and velocity gradients in mice and rats small intestines

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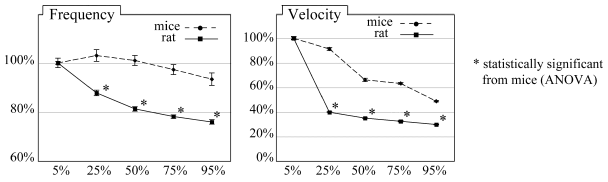
The frequency gradient of the slow wave along the small intestine has been described in large animals since Alvarez' first observations. There is no information on the frequency and velocity gradients in small animals. In this study, we have measured the frequency and velocity gradients in the intact small intestines of mice and rats.

Following chloroform anesthesia, the small intestines of 10 mice and 10 rats were removed in toto and positioned in an organ bath. The organs were superfused at 100 ml/min with Tyrode solution. Linear arrays of 10-16 extracellular electrodes were used to record slow wave potentials at 5 sites along the small intestine: at 5%, 25%, 50%, 75% and 95% of the total length. All recordings were made after a 30 minute control period. From the signals, the frequencies (c/min) and velocities (cm/sec) of 10.7 (±1.5) slow waves at every site were determined.

The results (percentages; average ± SD) are as follows:

In summary, the slow wave frequency gradient in rats is much larger than that in mice. Furthermore, in mice, the velocity of the slow wave declines gradually while in rats, the decline is larger and predominantly in the first part of the small intestine. These significant differences in slow wave gradients may induce differences in the pattern of intestinal contractions in these two species.



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Rhythmic postprandial patterns of contraction *in vivo* explained by simulated spatio-temporal maps

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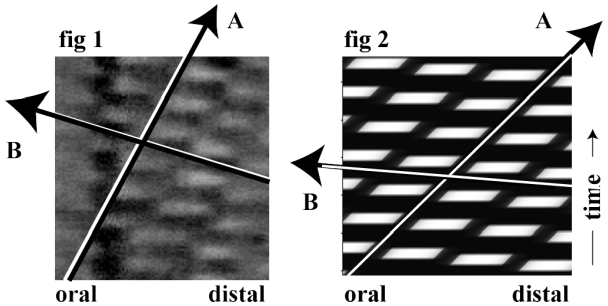
Previous experiments using videofluoroscopy image analysis showed slow wave driven peristalsis in mice. However, we also observed complex post-prandial (likely mixing) motor patterns in the small intestine and it was difficult to discern individual contractions in these spatio-temporal maps (fig 1). Comparing real-life ST-maps with simulated ST-maps could increase our understanding of such complex contraction patterns.

NIH Swiss mice were gavaged with contrast medium and the progress of the contrast in the small intestine was recorded fluoroscopically. Video images were digitalized at 10 frames/sec for 3 minutes, and transformed into ST-maps (fig 1). A simulation program (Stsim) was written (in RealBasic) to create different patterns of contractions and to convert these into simulated ST maps.

The approach is to simulate different patterns of contractions by varying frequency, propagation direction and velocity of contraction, convert them into ST-maps (fig 2) and to compare those with real-life ST-maps (fig 1). In the example shown below, post-prandial pattern of contractions was best mimicked by simulating segmental occlusion that slowly propagated in the aboral direction (line A). Furthermore, the contractions did not occur simultaneously but one after the other, progressing in the retrograde direction (line B). The length of the contracted segment was approximately 70% of the slow wave wavelength.

In summary, construction of simulated spatio-temporal maps provides a tool to understand, teach and analyze propagation patterns and their corresponding ST-maps.



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The impact of bolus volume on the characteristics of small intestinal intra-luminal impedance

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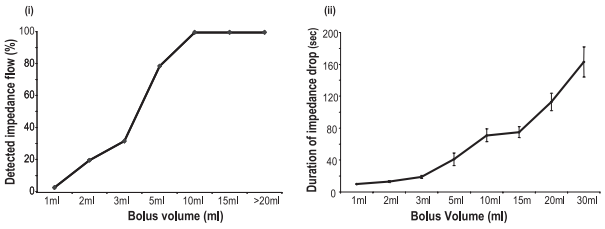
Although intraluminal impedance has recently been used to assessment intestinal flow and clearance, there are no data on relationship between the bolus volume and the characteristic of impedance signal. **Aim** To assess the impact of bolus volume on the characteristics of small intestinal impedance signals. **Methods** Concurrent small intestinal manometry-impedance measurements were performed on 8 healthy volunteers to assess the pattern of proximal jejenal fluid bolus movement over a 14cm-segment. A feeding tube was placed immediately above the proximal margin of the studied segment. During the motility-quiescent period, each subject was given 40 boluses of normal saline (0.9%), via the feeding tube, with a gradual increased in volume from 1ml to 30ml. A flow event was considered to be related to the bolus if there was > 12% impedance drop from baseline, over ≥ 3 consecutive segments within 10sec of bolus injection. Minor or major impedance flow events were defined as a duration of impedance drop < 60 or ≥ 60sec. Data compared with Chi square test and Pearson's correlation. **Results** The minimum volume required for a detectable SI impedance flow event was 2ml. A direct linear relationship between the SI bolus volume and the occurrence of impedance flow events was noted until SI bolus volume reached to 10ml, a volume which always produced an impedance flow event (**Figure**). There was a moderate correlation between the bolus volume and the duration of impedance drop (r= 0.63; P<0.0001; **Figure**) and the number of propagated channels (r=0.50; P<0.0001). High volume boluses were associated with more major impedance flow event (≥ 10ml boluses = 63%, 3ml boluses = 17%, and <3m boluses=0%; P=0.02). **Conclusions** There is a strong relationship between the volume of the boluses and changes in small intestinal impedance signals. A threshold volume of 2ml is required to produce a flow event. Bolus volume had an impact on both the type and the length of propagation of flow events



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Intravenous administration of cholecystokinin-8 (CCK-8), but not glucagon-like peptide-1 (GLP-1), suppresses ghrelin and stimulates peptide YY (PYY) release in healthy men

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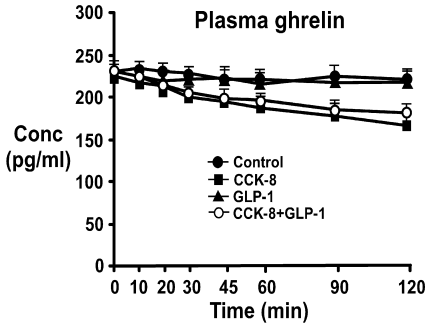
Background: The intestinal peptides, CCK, PYY and GLP-1, are secreted in response to meals. In contrast, ghrelin is released before, and suppressed following, eating. In humans, little is known about interactions between these peptides released from proximal and distal regions of the upper gastrointestinal (GI) tract. **Aim:** We investigated the effects of CCK-8 and GLP-1 (given alone or in combination) on plasma ghrelin and PYY. **Methods:** 9 healthy males (age 22±1 yrs; BMI 23±0.5 kg/m^2) were studied on four separate occasions, on which they received iv infusions of (1) saline (control), (2) CCK-8 (1.8 pmol/kg/min), (3) GLP-1 (0.9 pmol/kg/min), or (4) CCK-8+GLP-1 (combination of (2) and (3)), each for 120 min, in randomized, double-blind fashion. Plasma concentrations of total ghrelin and PYY were measured repeatedly by radioimmunoassays. **Results:** Baseline plasma ghrelin and PYY did not differ between days. Compared with control, plasma ghrelin (**Figure**) was markedly suppressed during infusion of both CCK-8 (baseline: 227.0±13.8 pg/ml, 120 min: 168.7±13.1 pg/ml, P=0.001) and CCK-8+GLP-1 (baseline: 231.3±7.3, 120 min: 183.1±11.1, P=0.001), with the effect of CCK-8+GLP-1 slightly less than that of CCK-8 between 60-120 min (P<0.05), while GLP-1 alone had no effect. Plasma PYY was stimulated by CCK-8 (baseline: 198.8±12.1 pg/ml, 120 min: 364.4±37.3 pg/ml; P=0.001) and CCK-8+GLP-1 (baseline: 201.9±15.7, 120 min: 218.6±15.7, P<0.01), but the effect of CCK-8+GLP-1 was significantly less than that of CCK-8 alone between 20-120 min (P<0.001), while GLP-1 alone had no effect. **Conclusions:** In healthy subjects, (1) exogenous CCK-8, but not GLP-1, suppresses ghrelin, and stimulates PYY, secretion, while GLP-1 appears to reduce the effects of CCK-8, and (2) the effects of CCK-8 on ghrelin and PYY release further support the concept that interactions exist between GI hormones released from proximal and distal regions of the upper GI tract. Postprandial release of CCK might, therefore, mediate, directly, or indirectly through PYY, the suppression of ghrelin after eating.



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Non-digestible capsule (SmartPill) as a novel diagnostic tool for detecting motility impairment within the gut

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Background: The unique features within an intraluminal environment (changes in pH and contractility) could help to identify the transition points along the alimentary tract that could serve as a basis for the measurement of GI transit times. **Aim:** To compare the results captured by a non-digestible capsule (SmartPill) in regards to small bowel transit time (SBTT) and colon transit time (CTT) among healthy subjects and gastroparetics. **Methods:** 23 patients with GP, 12 diabetics (DM) and 11 idiopathic (ID) 16F, mean age of 45, 20-66 range and 55 asymptomatic controls (C) 21F, mean age of 32, 19-57 range, participated in this multicenter study. After an overnight fast all subjects swallowed the capsule equipped with sensors measuring pressure, pH and temperature with data being collected by a portable recording device. The time between ingestion of capsule and the rapid, sustained rise of and at least 3 pH units from baseline and exceeding pH 4.0 was defined as GRT. Later on, a sudden drop of more than 1 pH unit for longer than 5 min. was regarded as an ileo-cecal (I-C) arrival. By subtracting GRT from I-C junction arrival time, SBTT was calculated, and in turn CTT was then able to be determined. Mann-Whitney and t-test were used for statistical analysis. Time was expressed in minutes. **Results:** In GP the median SBTT was not significantly longer than in C (300 vs. 281, NS). Also the SBTT between DM and ID was similar (mean ±SD: 294±92 vs. 312±165, NS). Median CTT in patients with GP was significantly longer than in C (1507 vs. 1026, P<0.001). Both subgroups of GP (DM & ID) also exhibited significant differences in terms of CTT versus C (median: 1623 vs. 1026, P=0.003; 1507 vs. 1026, P=0.043, respectively). The GP DM and ID differed numerically in CTT but it was not statistically significant (mean ±SD: 2625±1992 vs. 1595±651, NS). **Conclusions:** 1. The significantly longer CTT in patients with DM and ID gastroparesis suggests a neural impairment that could also include colon dysfunction; 2. SmartPill may have an important clinical role for diagnosis of abnormal motility in different parts of the gut as well as monitoring therapeutic interventions.

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The pancreatic polypeptide family and the migrating motor complex of the rat: Differential effects in the duodenum and jejunum

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Aim: To investigate the effects and receptor involvement of members of the pancreatic polypeptide family on migrating myoelectric complexes in rats in vivo. **Methods:** Rats were supplied with bipolar electrodes at 5 (duodenum), 15 and 25 cm (jejunum) distal to pylorus for electromyography of small intestine. The natural ligands neuropeptide Y, pancreatic polypeptide, peptide YY1-36 and peptide YY 3-36 were infused IV at doses of 0.5-400 pmol kg^{-1} min^{-1} for 60 min. The mechanisms of action were studied after pre-treatment with N^{ω}-nitro-L-arginine (L-NNA) 1 mg kg^{-1}, guanethidine 3 mg kg^{-1} and in bilaterally vagotomised animals. **Results:** PP inhibited myoelectrical activity dose-dependently in both the duodenum (ED50 5.8 pmol kg^{-1} min^{-1}) and jejunum (2.6 pmol kg^{-1} min^{-1}). PYY1-36 and PYY3-36 also had inhibitory effect in the jejunum (4.4 and 130 pmol kg^{-1} min^{-1}, respectively). PYY1-36 did not have any significant effect in the duodenum, whereas PYY3-36 stimulated myoelectrical activity at the highest doses. NPY was without effect. In the jejunum neither L-NNA, guanethidine or vagotomy had any significant influence on the inhibitory effects of PP, PYY1-36 and PYY3-36. In the duodenum, the effect of PP was inhibited by guanethidine but not L-NNA or vagotomy. The stimulatory effect of PYY3-36 in the duodenum was blocked by L-NNA and vagotomy, whereas guanethidine was without effect. **Conclusion:** PP and the two PYY peptides PYY1-36 and PYY3-36 have differential effects in the duodenum and jejunum. PP exerts a general inhibition of myoelectrical activity. Compared to PYY1-36, PYY3-36 has less potent inhibitory activity in the jejunum and stimulated duodenal myoelectrical activity. PYY1-36 had no significant effect in the duodenum.

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Alvimopan opioid antagonism modulates postoperative ileus

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Introduction: Neurogenic, inflammatory and pharmacological mechanisms are known to participate in causing postoperative ileus. Postsurgical opioid analgesics prolong ileus through neurogenic ''dysinhibition'' by blocking the release of inhibitory neuronal NO (Bauer 1991). However, opioids have also been shown to alter the release of nitric oxide from iNOS upregulated cultured macrophages. Our objective was to investigate the therapeutic potential of opioid antagonism in abrogating postoperative ileus through neuronal and inflammatory mechanisms. **Methods and Results:** To investigate the acute neural effects, Alvimopan and morphine were given 30 min and 0 min, respectively, prior to the 90 min gastrointestinal transit analysis period. In controls, morphine (3mg/kg, s.c.) delayed transit, which could be prevented by prior injection of Alvimopan (10mg/kg, s.c.) as measured with non-digestible FITC-labeled dextran fed orally 90 minutes before sacrifice. Laparotomy and surgical manipulation (SM) of the murine intestine resulted in a delay in gastrointestinal transit after 24 hours that tended to worsen by administrating exogenous morphine. Alvimopan only moderately improved transit in the SM animals. Laparotomy and light surgical manipulation (LM) caused a slight delay in transit which was further impaired with morphine. Similar to control transits, Alvimopan reversed the effect of morphine. To investigate a potential immuno-modulatory effect of these two opioid agents on the inflamed iNOS producing muscularis externa, the tissue was harvested from control and SM animals and organ cultured for 24 hrs in the presence and absence of the opioid agents. SM significantly increased NO secretion from the inflamed muscularis 38±1.5 fold over control. Interestingly, morphine tended to increase and Alvimopan significantly decreased NO released from the SM inflamed tissue (42±1.5 and 32±0.7-fold), with Alvimopan blocking the increase caused by morphine (36±1.6-fold)(N=8). **Conclusion:** Alvimopan blocks the pharmacological morphine-induced ileus and may also diminish exogenous and endogenous opioid modulation of inflammatory NO release.

	vehicles	Alvimopan + vehicle	Morphine + vehicle	Alvimopan + Morphine
Control	10.2±0.74	10.3±0.14	6.1±0.37	10.2±0.74
SM	4.3±0.30	4.8±1.14	3.9±0.08	5.6±1.27
LM	9.4±0.51	8.9±0.68	6.4±0.37	9.5±0.82

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Intestinal gas clearance correlates with the severity of intestinal motor dysfunction

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We have previously shown that intestinal gas propulsion is a sensitive parameter that is impaired in different conditions, including irritable bowel syndrome (IBS). The aim of the present study was to determine whether impaired gas propulsion correlates with the severity of intestinal dysfunction in different clinical conditions. **Methods.** In 15 patients with neuropathic-type intestinal dysmotility evidenced by manometry, in 15 patients with IBS and in 15 healthy subjects, intestinal clearance of an exogenous gas load was measured by infusing a mixture of gases (N_2 , O_2 and CO_2 in venous proportions) into the jejunum at 12 ml/min for 2 h, and measuring gas evacuation via an intrarectal cannula and abdominal symptoms by a 0-6 score scale. **Results.** Healthy subjects exhibited expeditous gas transit and cleared the gas load without intestinal gas retention (63±70 ml; N.S.) or abdominal symptoms (1.0±0.3; N.S.). By contrast, both in patients with IBS and with intestinal neuropathy gas transit was impaired, and both developed significant gas retention. However, gas retention was much more pronounced in patients with intestinal neuropathy (708±91 ml gas retention) than in patients with IBS (365±112 ml gas retention; $p<0.05$). ROC-curve analysis showed that a cut-off of 390 ml gas retention discriminated both groups of patients with a sensitivity of 67 % and a specificity of 80 %. Patients also developed significant abdominal perception during the test, but despite the differences in gas retention, similar levels of perception were scored by both groups of patients (3.3±0.6 score and 3.8±0.6 score, neuropathy and IBS, respectively; $p<0.05$ vs health for both). **Conclusion.** Intestinal gas clearance is a sensitive and reliable parameter of intestinal motor function, whose degree of impairment correlates with the severity of intestinal dysfunction.

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The regulatory roles of intrinsic nitrergic neurons in the peristalsis of the hamster ileum

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Nitric oxide (NO) is a potent smooth muscle relaxant and a critical inhibitory neurotransmitter in the gastrointestinal tract. Inhibitory neurotransmitters participate in peristalsis by promoting the descending inhibitory reflex, which facilitates propulsion of intraluminal bolus. Thus, it is expected that the inhibition of these reflexes would suppress intestinal peristalsis. However, it has been demonstrated that NO synthase (NOS) inhibitors enhance the frequency of peristalsis and the velocity of propulsion, shorten the latency of peristaltic wave initiation, and reduce the threshold volume needed to initiate peristalsis. These results suggest that intrinsic nitrergic neurons might have various actions in regulating peristalsis. Considering that the major function of peristalsis is the propulsion of intraluminal contents, measurement of the net propelled amount by peristaltic movements may be useful for evaluating neural basis of the intestinal

peristalsis. In this study, to investigate the regulatory roles of NO in the intestinal peristalsis, we have combined a method to measure the propelled volume with those to record intraluminal pressure and frequency of peristaltic movements. The oral and aboral ends of the dissected hamster ileum were attached to cannulas fixed horizontally. The volume of the liquid outflow from the aboral side was measured with an electric balance, and changes in intraluminal pressure were simultaneously recorded using a pressure transducer that were connected to the outflow line via a T connector. The application of intraluminal pressure by raising the level of liquid in the bottle connected to the oral end evoked peristaltic movements and intermittent outflow of the intraluminal liquid. The inhibition of intrinsic neurons by tetrodotoxin stopped propulsion, indicating that the liquid propulsion was correlated with neuron-regulated peristalsis. In good agreement with previous studies, a NOS inhibitor increased the frequency of peristaltic waves, but did not significantly influence the amplitude of intraluminal pressure change. In contrast, the volume of liquid propelled by one peristaltic movement was decreased much, leading to a reduced propelled volume per 5 min despite the enhanced frequency. It is therefore concluded that the intrinsic nitrergic neurons may increase efficiency of peristaltic movements of intestine.

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Cholinergic and tachykinergic co-neurotransmission in ''on'' and ''off'' contractions in human sigmoid colon

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Aim: To characterize the neurotransmitters mediating contractile responses induced by activation of excitatory enteric motor neurons (EMN) in human sigmoid colon. **Methods:** 51 circular smooth muscle strips (3x10 mm) of human sigmoid colon from 27 patients undergoing surgery for rectal cancer were studied in organ baths. **Experimental Design:** (1) Pharmacologic effect of the agonist acetylcholine (ACh, 0.01-100 μM) and substance P (SP, 0.001-10 μM). (2) Stimulation of EMN by electrical field (EFS 1-40Hz, 10s) and characterization of the contractions with atropine, the NK_2 receptor antagonist Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 (NK-2ra) and the $P2Y_1$ receptor antagonist MRS 2179. **Results:** 1) Both ACh and SP caused a concentration-dependent contraction in sigmoid strips that was antagonized by atropine (1μM) and the NK-2ra (1μM) respectively, and not affected by TTX (1μM) or MRS 2179 (10μM). 2) EFS induced two patterns of contractions: ''on''-contractions during EFS in 56% strips and ''off''-contractions following latency in 44%, both abolished by TTX. 2a) Atropine reduced amplitude of ''off''-contractions (9.7±1.7 vs. 4.8±1.6 g; P<0.05) and did not affect latency (17.9±0.6 vs. 16.1±0.1 s; NS). The NK-2ra also reduced amplitude of EFS-''off'' contractions (16.2±4.5 vs. 7.3±3.2 g; P<0.001). L-NNA (1mM) increased amplitude (7.9±1.7 vs. 10.4±2.1 g; P<0.05) and decreased latency (17.5±1.2 vs. 13.2±2.4s; P<0.001) of ''off'' contractions, further reduced by MRS2179 (1.5±0.4 s; P<0.001). 2b) Atropine reduced amplitude (11.5±1.4 vs. 5.2±1 g; P<0.05) and enhanced latency of ''on''-contractions (1.5±0.3 vs. 14±1.5 s; P<0.05). The NK-2ra also significantly decreased amplitude of ''on'' contractions (11.1±2.2 vs. 4.7±0.6 g; P<0.05). Sequential addition of NK-2ra and atropine almost abolished amplitude of ''on''-contractions at all frequencies of EFS. 2c) MRS 2179 reduced amplitude of ''off'' (8.5±2.2 vs. 6.3±2 g; P<0.001) and ''on'' contractions (2.5±0.6 vs. 1.4±0.3g P<0.05). **Conclusions:** EFS-''on'' and ''off'' contractions in human sigmoid colon are caused by stimulation of excitatory EMN co-releasing ACh acting on muscular muscarinic receptors and tachykinins acting on NK_2 receptors. Latency is due to inhibitory nitrergic and purinergic (trough P2Y1 receptors) co-neurotransmission. $P2Y_1$ receptors might modulate prejunctionally excitatory pathways to human colon. (Support: SGR 2005/00255 and Laboratorios Menarini SA).

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Effects of mitochondrial inhibitors and temperature modulation on rhythmic electrical activity and calcium transients in guinea pig gallbladder smooth muscle

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Gallbladder smooth muscle (GBSM) exhibits rhythmic, spontaneous action potentials (APs) that are elicited by Ca^{2+} influx via voltage dependent Ca^{2+} channels (VDCC), and depend on sarcoplasmic reticulum (SR) Ca^{2+} release via 1,4,5-inositol trisphosphate ($Ins(1,4,5)IP_3$) receptors. Studies in ICC and other types of smooth muscle suggest that Ca^{2+} mobilization, involving both SR and mitochondria, is fundamental for pacemaking and propagation of rhythmic electrical activity, and that these events are temperature dependent. The cellular mechanisms that underlie the distinctive excitability of the gallbladder, which is a tonic organ characterized by phasic activity of individual muscle bundles, are not well understood. We therefore investigated actions of mitochondrial inhibitors on APs, Ca^{2+} flashes, and Ca^{2+} waves in intact muscularis whole mount preparations using standard microlectrode recording of membrane voltage and laser confocal imaging of Ca^{2+} transients in Fluo-4 AM-loaded tissues. Intact preparations were also used to study the effect of temperature modulation on APs. The protonophores FCCP and CCP (1 μM each), which collapse mitochondrial membrane potential, rapidly increased the frequency of APs, Ca^{2+} flashes, and Ca^{2+}waves before abolishing these events within 3-10 minutes. Rotenone and antimycin A mixture (10 μM each), inhibitors of the respiratory chain complex, significantly reduced the frequency of APs. These agents, as well as the mitochondrial Ca^{2+} uptake inhibitor, Ru 360 (10 μM), and a mitochondrial Na^{+}-Ca^{2+} exchange inhibitor, CGP 37157 (30 μM), reduced Ca^{2+} flashes and Ca^{2+} waves within 10-30 minutes. Reducing metabolic activity by lowering temperature to 21-23 degrees Celsius (room temperature) eliminated APs. When raising temperature gradually from room temperature to 37 degrees, APs first appeared at ~ 24 degrees. At temperatures in the range of 25-28 degrees, the frequency of APs increased, but the durations of the spike and plateau phases of the AP decreased with increasing temperature. These durations were stable from 28-37 degrees while the APs frequency was constant between 32-37 degrees. The resting membrane potential, amplitudes of APs, spikes and plateau of APs, and the average slope of the upstroke depolarization were not affected by temperature change. These results indicate that in GBSM mitochondrial Ca^{2+} handling and metabolic activity modulate the excitability, and possibly pacemaking activity, and are thus likely to influence gallbladder motility. Supported by NIH grants NS 26995.

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Characterization of inward currents in human colonic circular smooth muscle cells

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Background: Colonic smooth muscle contractile activity requires coordinated activity of several ion channel types. However, little is known about the ion channels that contribute to inward current in human colonic circular smooth muscle cells. The aim of this study was to determine the ionic selectivity and identity of the channels that contribute to the inward current. **Methods:** Single human colonic circular smooth muscle cells were obtained from colonic tissue resected for colon cancer. Inward current was measured by whole-cell patch clamp technique with Cs^{+} in the intracellular solution. Nifedipine (1 μm) was used as an L-type calcium channel blocker and mibefradil (0.3 μm) used as a T-type calcium channel blocker. RT-PCR was carried out on RNA isolated from circular smooth muscle strips. **Results:** Currents were recorded from 19 cells. The inward current had two main components, a fast inactivating component

(approximately 10s of ms) and a slower component that inactivated over 100s of ms. In 17 of the 19 cells both components were present. The slow component was blocked by nifedipine (1 μm, n=15, −51pA to −13pA) and abolished when extracellular Ca^{2+} was replaced with Mn^{2+} suggesting it was carried by L-type Ca^{2+} channels. The fast component of the inward current was not blocked by nifedipine (n=15) or by replacement of Ca^{2+} with Mn^{2+} (n=4). Mibefradil (0.3 μM, n=4) had no effect on the size or kinetics of the fast component of the inward current. Replacement of Na^+ with NMDG abolished the fast current (−64pA to −4pA, n=9) suggesting it was carried through cation permeable channels. Replacement of the NaCl Ringers solution with KCl Ringers solution (Na^+ free) completely abolished the fast component in 4 cells but had little effect in 5 other cells suggesting that, in different cells, the fast component was made up of two separate currents carried by different channels, one selective for Na^+ and the other a non-selective cation channel. The selective Na^+ channel blocker QX314 (500 μm) inhibited the fast component of the inward current in 4 cells but had no effect on 1 cell. RT-PCR detected message for SCN5A, the alpha subunit of $Na_v1.5$. **Conclusion:** These data suggest the presence of at least three cation channel types carrying Na^+ and Ca^{2+} into smooth muscle from the circular layer of the human colon and contributing to regulation of human colonic contractility; an L-type Ca^{2+} channel, a Na^+ selective channel, likely encoded by $Na_v1.5$, and a non-selective cation channel. Supported by NIH DK 57266 and DK 57061.

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Expression of telethonin in human GI smooth muscle

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Background: There is increasing evidence that contractile pathways in different muscle cell types are more conserved than previously thought. Cardiac myocytes and gastrointestinal smooth muscle cells both express SCN5A and both require syntrophin for the full expression of the Na^+ current. **Aim:** Determine if contractile proteins associated with the Z-disc in cardiac myocytes are also expressed in gastrointestinal smooth muscle. Our initial studies focused on telethonin, as telethonin is a critical cytoskeletal element that links the sarcomeric cytoskeleton to the membrane-associated cytoskeleton. It is a substrate for contractile proteins associated with the Z-disc, and is required for muscle differentiation. **Methods:** Reverse-transcription polymerase chain reaction (RT-PCR) and immunohistochemistry were used to determine expression of telethonin in human circular and longitudinal intestinal smooth muscle cells. Control experiments were performed by omitting the primary antibodies and by using pre-immune serum. **Results:** RT-PCR was carried out on RNA extracted from each muscle layer separated by dissection. Telethonin was expressed in both the jejunal muscle layers. To locate expression of telethonin immunohistochemistry experiments were performed using multiple polyclonal antibodies raised against human telethonin. A similar pattern of distribution of telethonin was observed in both the longitudinal and the circular muscle layers. Together with a diffuse staining of muscle cells, individual muscle cells also showed a striated pattern of staining. This striated pattern staining outlined also the nuclei of the muscle cells. The perinuclear staining was also confirmed by confocal microscopy. Control experiments performed by omitting the primary antibodies and by using pre-immune serum showed no staining. **Conclusion:** Together with the known expression of telethonin in striated and cardiac muscle it appears that telethonin is also expressed in gastrointestinal smooth muscle. Its role in smooth muscle contractile events remains to be determined. Supported by NIH DK52766 and DK57061, and Telethon Foundation, Italy (Grant GGP04088 to G.F.)

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Melatonin improves acute cholecistitis-induced gallbladder motility disorders

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Impaired smooth muscle contractility is a hallmark of acalculous cholecystitis (AC). There is evidence indicating that melatonin can ameliorate the harmful effects of inflammation in the gastrointestinal tract. In this work, we investigated the possible benefits of melatonin treatment on myogenic and neurogenic gallbladder contraction in AC. AC was induced by 2 day bile duct ligation and the functional responses to agonists and nerve stimulation were assessed by performing isometric tension recordings in gallbladder strips. Electrical field stimulation (EFS)-evoked contractions were significantly reduced in AC strips at all frequencies tested (70–80 % of reduction). These contractions were TTX, atropine and L-name insensitive but were partially blocked by capsaicin and ω-conotoxin. In addition, the myogenic responses to KCl, capacitative calcium entry, ACh, CCK and ionomicin were also impaired in AC, suggesting that both nerve and smooth muscle degeneration contribute to the AC-induced motor dysfunction. Melatonin treatment (30 mg/Kg/day) for 12 days did not improve the motor pattern of AC gallbladder strips but was able to normalize Ca^{2+} handling in AC gallbladder smooth muscle cells. When bile duct was de-ligated for 2 days after inducing AC the motor impairment of the gallbladder worsened, particularly the neurogenic contractions (93–95 % of reduction). Under these conditions, melatonin treatment (30 mg/Kg/day for 8 days before inducing AC, during AC and 2 days after deligation) caused a significant recovery of the gallbladder motility. Thus, myogenic contractions induced by ACh, capacitative calcium entry, KCl and ionomicin were similar to those in control tissue. Although melatonin treatment enhanced by 50% the contractile response to CCK, this was still impaired compared to control strips (60 % of reduction). There was also an improvement in the neurogenic contractions that restored the sensitivity to TTX, and atropine. The normalization of the contractility was also companioned by the reestablishment of Ca^{2+} signalling. These findings indicate that melatonin treatment protects gallbladder against AC-induced damage and suggest that melatonin may be considered a potentially useful therapeutic agent for treating gallbladder inflammation. Supported by MEC (BFU 2004–0637) and JEX (2PR03A020).

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Altered electrical activity in duodenal muscle cells from *mdx* (dystrophic) mice

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Our laboratories are currently interesting in studying differences in gastrointestinal motor function between normal and *mdx* mice, mutant animals for the gene encoding dystrophin, a cytoskeletal protein localised at the inner face of the plasma membrane in cells, including the intestinal muscle cells, the enteric neurones and the interstitial cells of Cajal (ICCs). Lack of dystrophin, in *mdx* mice, is accompanied by changes in the ICC ultrastructure and in the functionality of intestinal segments, such as stomach and proximal colon. Since our previous studies have shown also duodenal contractility disturbances in *mdx* mice, the aim was to compare the intracellular electrical activity of duodenal circular muscle cells between normal and *mdx* mice. *Normal animals:* Duodenal circular smooth muscle cells showed ongoing discharge of biphasic slow waves with an amplitude waxing and waning and a frequency ranging between 36 and 45 waves min^{-1} (mean 41.1 ± 0.9 waves min^{-1}, n=10). The highest slow waves showed an amplitude varying between 8 and 10 mV (mean, 9.3 ± 0.3 mV, n=10) and a rate of rise of 37.5 ± 4.1 mV sec^{-1}. The membrane potential at its most negative value (the resting membrane potential, RMP) ranged between –52 and –68 mV (mean, −63.9 ± 2.0, n=10). *Mdx mice:* RMP ranged between –39 and –58 mV (mean, 45.6 ± 2.3 mV, n=10), being significantly different from that of normal mice ($P<0.05$).

Slow waves were larger and with a higher frequency than in normal animals. Their amplitude varied between 14 and 18 mV (mean, 16.4 ± 0.5 mV, n=10), their frequency between 43 and 58 waves min^{-1} (mean 51.6 ± 1.8 waves min^{-1}, n=10). The majority of the slow waves showed an initial rapid upstroke with a rate of rise of 70.6 ± 5.7 mV sec^{-1} and a less evident waxing and waning of their amplitude. Atropine (1 μM), guanethidine (1 μM), the neuronal blocker TTX (1 μM) or the nitric oxide synthase blocker L-NAME (100 μM) had no influence on RMP, slow wave rhythmicity or amplitude in both preparations. In conclusion, in *mdx* duodenal circular muscle cells there are differences in the electrical activity that match the observed differences in the motor pattern, i.e. sustained mechanical tone and motor hyperactivity. A primary dysfunction in non-neural cells, likely in the ICCs, responsible for the altered rhythmicity, might be speculated.

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Smooth muscle cells isolated from normal and inflamed human colon: TLR4 expression and morphofunctional features

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Mucosal inflammatory conditions likely lead to an exposure of underlying tissues to luminal bacterial antigens. Among them, bacterial lipopolysaccharide (LPS) is known to suppress smooth muscle contractility. LPS is recognized by Toll-like 4 receptors (TLR4) that have been shown to be expressed by various intestinal cells, included myofibroblasts, and to be significantly increased in intestinal mucosa in association with inflammatory bowel diseases. The expression of TLR4 and the effects of its direct activation by LPS, however, have never been investigated in colonic smooth muscle. Aim of this study was to evaluate TLR4 expression and activation in smooth muscle cells (SMC) isolated either from normal or inflamed (ulcerative colitis) human colon. TLR4 expression was tested by RT-PCR and immunohisto-chemistry using a specific biotinated TLR4 antibody (Mouse IgG HTA 125, BD Biosciences) on a highly pure primary SMC suspension. LPS was obtained from the pathogen strain of Escherichia coli O111:B4 (Vinci-Biochem). On normal SMC, the presence of TLR4 was rather low and progressively increased on cell surface following exposure to LPS indicating an inducible expression of these receptors on SMC. In parallel, LPS exposure (1microgram/ml) caused morphological and functional alterations of these cells that consist in a time and dose-dependent cell shortening and an inhibition of acetylcholine-induced contraction. After 60 min LPS-exposure, cell length was decreased by 19.5±2.7% whereas contractile response by 73.3±3.1%. Both effects were reduced by the NFkB inhibitor MG132 indicating an involvement of this intracellular signalling pathways in inducing such alterations. On the contrary, in SMC isolated from inflamed colon, TLR4 resulted constitutively highly expressed. In respect to "normal" SMC, cells isolated from inflamed colon resulted to be shorter (33% decrease in resting cell length) and presented a reduced response to contractile agonists (20% inhibition in acetylcholine-induced maximal contraction). In conclusion, bacterial components directly modify morpho-functional features and TLR4 expression of human colonic SMC. A better understanding of the distinct SMC phenotype in inflamed colon might extend our current knowledge on the pathogenesis of bacterial-related motor disorders.

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Effect of NaHS on resting membrane potential of circular smooth muscle cells in mouse and human small intestine and colon

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Hydrogen sulfide (H_2S) has been proposed as the third gaseous modulator/transmitter. Endogenous production of H_2S from cysteine occurs in tissues through the activity of two enzymes, cystathionine-γ-lyase and cystathionine-β-synthetase. Both are found in the gastrointestinal tract (Hosoki, R. et al., Biochem Biophys Res Commun 237:527–531, 1997). Exogenously added H_2S relaxes circular muscle of the guinea pig and rabbit ileum (Hosoki, R. et al., Biochem Biophys Res Commun 237:527–531, 1997; Teague, B. et al., Br J Pharmacol 137:139–145, 2002). In this study, we tested the effect of exogenous H_2S on the resting membrane potential (RMP) of circular smooth muscle cells of the mouse and human small intestine and colon. Sharp glass microelectrodes were used to record RMP of circular smooth muscle cells. Sodium hydrogen sulfide (NaHS), a donor of H_2S, was applied locally close to the recorded cells. Values for RMP are given as mean ± SE. In mouse small intestine, NaHS (1 mM, 0.1 ml) evoked a depolarization that was 6.0±1.4 mV in amplitude and 387±127 sec in duration (n=9 cells from 4 preparations). In mouse colon, NaHS (1 mM, 0.1 ml) evoked a hyperpolarization that was 6.7±0.9 mV in amplitude and 345±62 sec in duration (n=20 cells from 8 preparations). In human small intestine, NaHS (2 mM, 0.1 ml) evoked a hyperpolarization that was 4.5±0.7 mV in amplitude and 210±76 sec in duration (n=14 cells from 3 preparations). In human colon, NaHS (2 mM, 0.1 ml) evoked a hyperpolarization that was 3.6±0.7 mV in amplitude and 279±68 sec in duration (n=10 cells from 4 preparations). Pinacidil (50 μM) hyperpolarized the RMP by 25.5±1 mV (n=2 cells from 2 preparations), an effect that was blocked by the K_{ATP} channel blocker glybenclamide (20 μM), suggesting that glybenclamide is able to access K_{ATP} channels in the muscle strips. In the mouse colon, the hyperpolarization evoked by NaHS was not significantly (P>0.05) affected by glybenclamide (20 μM) (7.2±1.5 mV [n=3] vs 6.6±1.4 mV [n=6], respectively). We conclude that NaHS can modulate the RMP of circular smooth muscle cells of mouse and human intestine and that this effect is not due to activation of K_{ATP} channel current. Supported by NIH Grant DK 17632.

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α-SMA immunoreactivity in the normal human ileum: playing fool play?

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α-SMA is a major component of the contractile apparatus of smooth muscle cells (SMC). α-SMA immunoreactivity (-ir) has been extensively used to assess SMC distribution in tissue sections. Defect of α-SMA-ir has been reported as an abnormal feature in patients with chronic intestinal pseudoobstruction (CIPO) (Smith et al., 1992; Knowles et al., 2004). However, the recent claim that the circular muscle layer of normal human ileum lacks α-SMA-ir (Gamba et al., 2004; Jaynes et al., 2005) has shaken the confidence in the normal distribution of α-SMA-ir in the human ileum.

Routine paraffin sections of normal human ileum (18 cases) were collected from 3 academic hospitals. Immunohistochemistry for α-SMA and HDAC8, a novel smooth muscle marker (Waltregny et al., 2004) was replicated in 3 research laboratories. Staining was qualitatively assessed independently by 3 investigators. While an homogeneous pattern was usually observed, in roughly 1/3 of cases,? α-SMA-ir appeared strikingly weaker - but still clearly discernible - in the outer circular muscle layer, while other SMC in the same field consistently showed strong? α-SMA-ir. Antigen retrieval increased the intensity of α-SMA-ir in the outer circular muscle layer. In contrast to ?α-SMA-ir, HDAC8-ir was homogeneously distributed in all cases. One juvenile CIPO patient with hypoganglionosis of the myenteric plexus exhibited a lack of ?both α-SMA-ir and HDAC8-ir in the ileal outer circular muscle.

Although in our hands α-SMA-ir was always discernible in the outer circular layer on paraffin sections of the normal human ileum, it may sometimes appear faint, especially when antigen retrieval had not been performed. Thus caution should be exerted in the interpretation of an "abnormal" distribution of α-SMA-ir in the human ileum. HDAC8-ir appeared as a more consistent SMC marker. A genuine lack of SMC markers may exist in the outer circular muscle layer of the ileum in some CIPO patients.

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In-vitro study of colonic contractility after pharmacological and electrical field stimulation in patients with primary chronic constipation

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The aim of the study was to investigate the pathophysiological mechanisms underlying primary chronic constipation (PCC). Isolated circular and longitudinal smooth muscle strips obtained from patients with PCC (n=13) and from a control group (n=14) were evaluated on their response to cholinergic, adrenergic and NANC stimulation using electrical field stimulation (EFS). The contractile responses of circular muscle strips obtained by cholinergic stimulation and EFS were significantly lower in PCC compared to controls, while longitudinal muscle strips showed higher contractile response to acetylcholine. After cholinergic and sympathetic blockade (NANC conditions) and application of EFS the inhibitory responses were markedly weaker in PCC. Blockade of nitric oxide synthase with L-NNA resulted in similar reduction of the inhibitory response evoked by EFS. The findings illustrate that the circular muscle layer of patients with PCC is much less susceptible to cholinergic stimuli. Moreover, the relaxation caused by inhibitory NANC nerves is significantly reduced. However, the reduced effect of NANC innervation is not attributed to a deficiency of the nitric oxide biosynthesis. The data suggest that alterations of both cholinergic and NANC neurotransmission may play an important role in intestinal motor disturbances found in patients with PCC.

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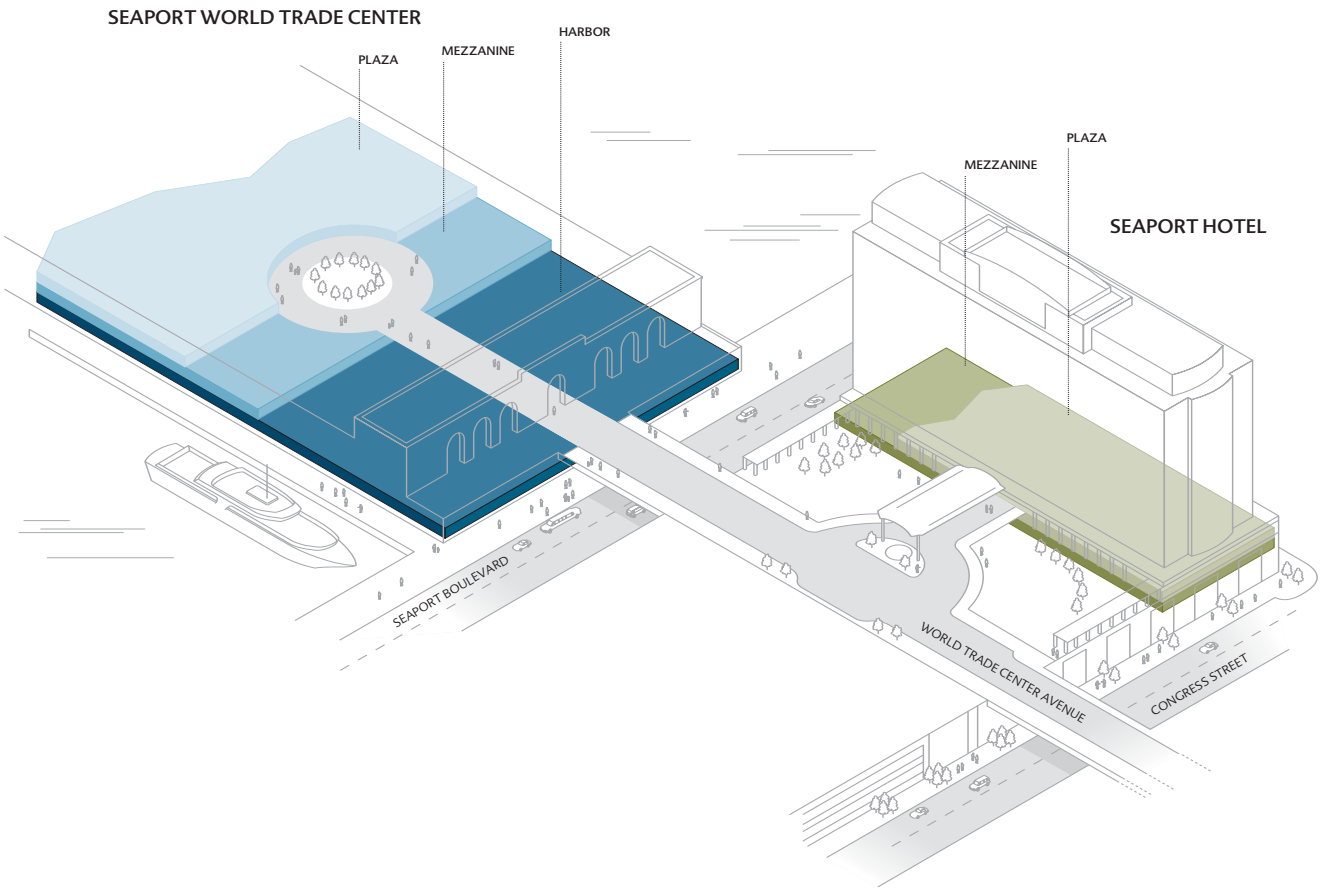

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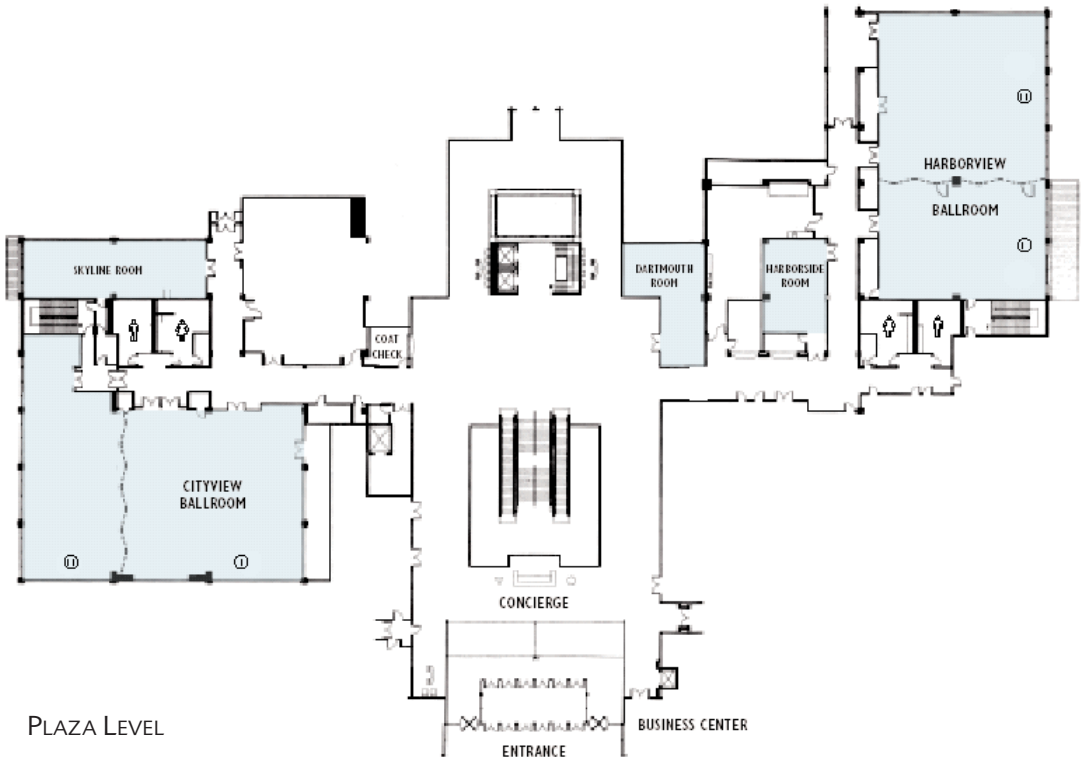

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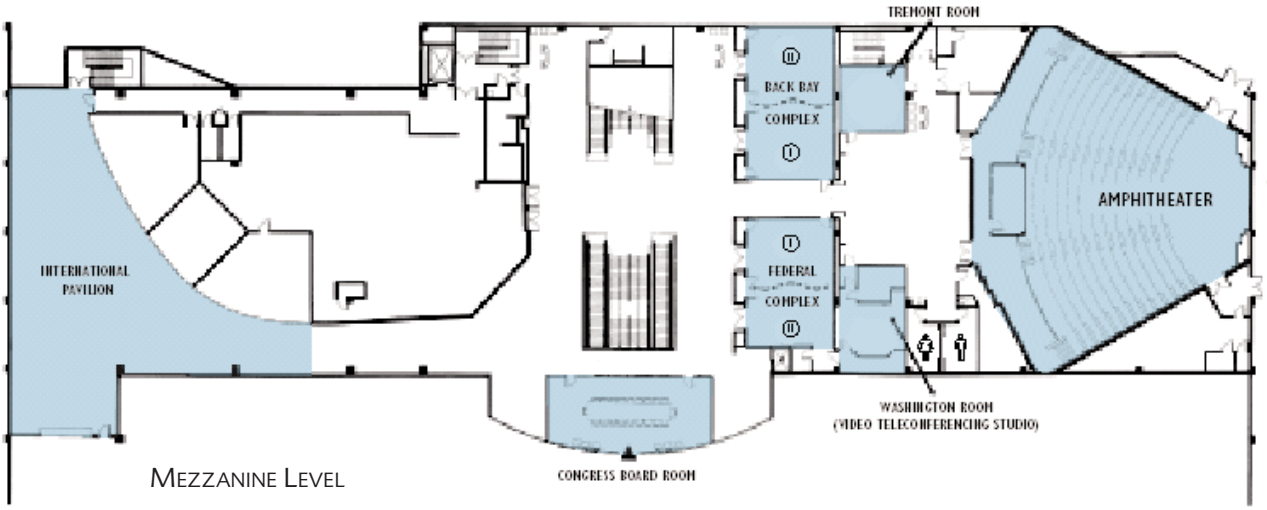

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FUTURE MEETINGS

2007 AMS Scientific Meeting on Diabetes and the Gut
Meeting Dates: March 1-4, 2007
Abstract Deadline: November 15, 2006
Location: The Sheraton and Westin Grand Bahama Resorts at Our Lucaya Resort and Convention Center, Freeport, Grand Bahama Island

Functional Brain-Gut Research Group Young Investigators' Conference
Meeting Dates: March 23-25, 2007
Abstract Deadline: January 15, 2007
Location: L'Auberge Del Mar Resort and Spa, Del Mar, California

21st International Symposium on Neurogastroenterology and Motility
Meeting Dates: September 2-5, 2007
Abstract Deadline: March 17, 2007
Location: Jeju, Korea, from
Hosted by the International Group for the Study of Gastrointestinal Motility and the Organizing Committee of the 21st ISNM
For further information log on to: www.isnm2007.org or www.ksgm.org/

7th International Symposium on Functional Gastrointestinal Disorders
Meeting Dates: April 12-15, 2007
Abstract Deadline: March 1, 2007
Location: Pfister Hotel, Milwaukee, Wisconsin

Neurogastroenterology and Motility 2008 Joint International Meeting
Meeting Dates: November 6-8, 2008
Location: Lucerne, Switzerland