DOI: 10.1111/nmo.13816

Neurogastroenterology & Motility NGM WILEY

Oral Presentations

19 | Gastrointestinal infection breaks tolerance to dietary antigens and triggers food-induced abdominal pain

Javier Aguilera-Lizarraga[1]; Maria Viola[1]; Lisse Decraecker[1]; Sales Ibiza[1]; Morgane Florens[1]; James Moon[2]; Alexandre Denadai-Souza[1]; Guy Boeckxstaens[1]

[1]*KU Leuven, Leuven, Belgium;* [2]*Harvard Medical School, Charlestown, USA*

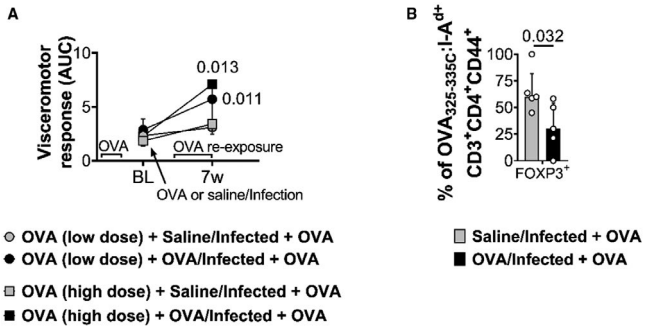
Background/Objective: Food ingestion is recognized as a major trigger of symptoms in irritable bowel syndrome (IBS). However, the mechanisms underlying food sensitivities in IBS, including the role of antigen-specific regulatory T cells (T_{regs}), key players in oral tolerance, are largely unknown. We previously showed in mice that an intestinal infection impairs the establishment of tolerance to food antigens, triggering local (colon) antigen-specific IgE production and mast cell-dependent visceral hypersensitivity (VHS) upon antigen re-exposure. Here, we investigated whether also established oral tolerance can be lost and is associated with changes in the immune response to food antigens.

Methods: Mice were tolerized to ovalbumin (low-dose: 1 mg/mL, 1 week, drinking water; or high-dose: 50 mg, 3 doses by oral gavage) 2 weeks prior to infection. Thereafter, mice were infected with *Citrobacter rodentium* in the presence/absence of ovalbumin. Five weeks later, mice were re-exposed to ovalbumin. Visceral sensitivity was assessed by recording the visceromotor response upon colorectal distension. Colon-draining mesenteric lymph nodes (coMLN) were isolated to identify ovalbumin-specific T cells using $OVA_{325\text{-}335C}$:I-A^d tetramers and to quantify IL4 release in response to $OVA_{325\text{-}335C}$ using ELISpot. Lastly, naïve mice were injected with monoclonal ovalbumin-specific IgE and were later gavaged with ovalbumin.

Results: Mice tolerized to ovalbumin prior to infection developed VHS upon ovalbumin re-exposure, but only if ovalbumin was present during the infection (Figure 1A). Ovalbumin ingestion recapitulated VHS in mice injected with ovalbumin-specific IgE, similarly to mice infected in the presence of ovalbumin. Remarkably, coMLN from VHS-mice showed lower ovalbumin-specific FOXP3$^+$ T_{regs} (Figure 1B) and an increment in IL4-producing cells compared to normosensitive mice. VHS was also associated with increased ovalbumin-specific GATA3$^+$FOXP3$^-$ and RORγT$^+$FOXP3$^-$ T cells in the coMLN.

Conclusion: Food-induced VHS following a gastrointestinal infection results from loss of tolerance to dietary antigens associated with a reduction in food antigen-specific T_{regs} and increased production of GATA3$^+$ IL4-producing cells and antigen-specific IgE, characteristic of a Th2-like response.

Figure 1. A, Visceromotor response to colorectal distention in BALB/c mice tolerized with OVA prior to *C. rodentium* infection: OVA (low dose) + saline/infected + OVA (n = 6, grey circles) and OVA (low dose) + OVA/infected + OVA (n = 8, black circles), OVA (high dose) + saline/infected + OVA (n = 6, grey squares) and OVA (high dose) + OVA/infected + OVA (n = 9, black squares). B, Quantification of CD3$^+$CD4$^+$CD44$^+$$OVA_{325\text{-}335C}$:I-$A^{d+}$FOXP3$^+$ OVA-specific T_{regs} in colon-draining mesenteric lymph nodes of OVA/saline + OVA (n = 5, grey bar) and OVA/infected + OVA (n = 5, black bar) mice. Two-way repeated analysis of variance (ANOVA) with Sidak's multiple comparisons test was performed for A and two-tailed Mann-Whitney test was performed for B. Data are shown as median ± IQR. AUC, area under the curve; BL, baseline; w, weeks; OVA, ovalbumin.



39 | Long-term functional outcomes in patients with Hirschsprung's disease with different lengths of the aganglionosis

Sanne Verkuijl[1]; Rob Meinds[2]; Monika Trzpis[1]; Paul Broens[1]
[1]*University of Groningen, University Medical Center Groningen, Groningen, The Netherlands;* [2]*Medisch Spectrum Twente, Enschede, The Netherlands*

Background/objectives: Little is known about the differences in long-term functional outcomes after different lengths of bowel affected by Hirschsprung's disease. The aim of this study was to compare the long-term functional outcome in patients with different lengths of aganglionosis of the colon due to Hirschsprung's disease.
Methods: This is a nationwide, cross-sectional study. All surgically treated patients, suffering from Hirschsprung's disease, older than 7 years were sent the Defecation and Fecal Continence Questionnaire. Additionally, pediatric patients were asked to fill in the Child Health Questionnaire Child Form 87 (CHQ-87), while adult patients completed in the World Health Organisation Quality of Life questionnaire 100 (WHOQOL-100). Patients who were unreachable, had a permanent stoma or with intellectual disability were excluded. The patients were categorized according to the length of the aganglionosis in three groups: rectosigmoid, long-segment or total colonic.
Results: For the analysis we included 336 patients, from which 83.9% suffered from aganglionosis limited to the rectosigmoid (n = 282). The type of surgical reconstruction, age at time of surgery and age at follow-up did not differ significantly between the three types. Constipation was significantly more prevalent in the rectosigmoid group in comparison to the long segment and total colonic group (24.1% vs 6.9% and 4.0%, P = 0.009, respectively). However, the prevalences of fecal incontinence, urinary incontinence and quality of life of both the CHQ-87 and WHOQOL-100 forms did not differ significantly between patients with the three different lengths of the colonic aganglionosis (Figure 1).
Conclusions: In contrary to the current thought, this study shows that fecal incontinence, urinary incontinence, and quality of life of patients suffering from long-segment and total colonic types of Hirschsprung's disease are comparable with the rectosigmoid type in the long run. Long-term constipation is more prevalent in patients with Hirschsprung's disease of the rectosigmoid.

42 | Illuminating central neurocircuitry underlying visceral pain via optogenetics in rats

Anthony Johnson[1,2]; Beverely Greenwood-Van Meerveld[1,2]
[1]*VA Health Care System;* [2]*University of Oklahoma Health Sciences Center*

Background: Psychological stress exacerbates abdominal pain experienced by patients with irritable bowel syndrome (IBS) through abnormal limbic activation in response to visceral stimulation. In experimental models, optogenetic tools allow direct modulation of central neuronal circuits in freely-moving animals. The specific objective of this study was to determine if optogenetic modulation of limbic circuitry would affect stress-induced colonic hypersensitivity in rats.
Methods: Bilaterally, neurons in the central amygdala (CeA) were infected with vectors expressing channelrhodopsin (ChR2) or halorhodopsin (HR3.0) and cannula were implanted at the bed nucleus of the stria terminalis (BNST) in male Fischer 344 rats. In one cohort, the role of the CeA-BNST circuit in colonic sensitivity was evaluated. A second cohort underwent 7 days of water avoidance stress (WAS) or SHAM-stress (1 h/d). Twenty-four hours after the last WAS/SHAM-stress, the role of the CeA-BNST circuit in stress-induced colonic hypersensitivity was evaluated. Colonic sensitivity was assessed as the visceromotor response to graded, isobaric colorectal distension (20-60 mm Hg) in freely moving rats. Results were analyzed with repeated measure, multifactor ANOVA with Bonferroni's post-hoc analysis (mean ± SD).
Results: In the first cohort, ChR2 mediated activation of the CeA-BNST circuit induced colonic hypersensitivity (60 mm Hg: 24.8 ± 4.9 vs 38.0 ± 8.4, P = 0.0004), whereas inhibition with HR3.0 did not affect colonic sensitivity (60 mm Hg: 24.6 ± 8.9 vs 27.8 ± 6.0, P = 0.33). In the second cohort, ChR2 activation in rats exposed to WAS did not exacerbate the stress-induced colonic hyperalgesia (60 mm Hg: 34.5 ± 12.5 vs 39.0 ± 10.0, P = 0.99). In contrast, HR3.0-mediated inhibition of CeA-BNST circuit normalized colonic sensitivity (60 mm Hg: 36.5 ± 9.6 vs 20.7 ± 6.4, P = 0.042). HR3.0 activation did not affect colonic sensitivity in SHAM-stress exposed rats.
Conclusions: Real-time manipulation of the CeA-BNST circuit with optogenetic tools is sufficient to change colonic sensitivity in freely moving rats. Thus, stress-induced colonic hypersensitivity can be induced and maintained by central limbic neurocircuits. These results provide support for developing therapies targeting limbic circuits to decease chronic stress-induced visceral pain.

69 | Sensory epithelial gastrointestinal enteroendocrine cells use different signaling pathways for chemo and mechanotransduction

Constanza Alcaino; Kaitlyn Knutson; Sara Whiteman; Vaishali Nayak; Halil Kacmaz; Peter R. Strege; Gianrico Farrugia; Arthur Beyder
Enteric NeuroScience Program (ENSP), Division of Gastroenterology & Hepatology, Department of Medicine, Mayo Clinic, Rochester, MN, USA

Background: Enteroendocrine cells (EECs) in the gastrointestinal (GI) epithelium are important specialized sensors of luminal forces and chemicals, like nutrients and bacterial metabolites. EECs regulate GI and systemic physiology by releasing a range of important signaling molecules, like serotonin and incretins. EEC force signal transduction mechanisms are important but remain poorly understood. EEC chemotransduction depends on the voltage-gated Ca^{2+} channel (Ca_V) isoforms L-, P/Q-, but not T-type. T-type Ca_Vs are highly

expressed in EECs and involved in mechanotransduction in other specialized mechanosensors, but their role in EECs is unknown.

Aim: Determine if T-type Ca_Vs (Ca_V3.2+) have a role in EEC mechanotransduction.

Methods: We created two mouse models by breeding NeuroD1-cre with RiboTag and tdTomato/GCaMP5 mice. We bred Ca_V3.2-Cre mouse with tdTomato to lineage-trace Ca_V3.2+ cells. We evaluated EEC Ca_V expression by RT-qPCR and immunohistochemistry (IHC). Mechano- and chemo-sensitivity in primary cultures was assessed by Ca^{2+} imaging with either membrane displacement or the Trpa1 channel agonist AITC.

Results: Purified NeuroD1+ EEC transcripts were enriched for epithelium markers (*Vil1*), EECs (*ChgA*), and Ca_V α-subunits: L- (*Cacna1a*), P/Q- (*Cacna1d*) and T-type (*Cacna1h*) (n = 3, $P < 0.05$). IHC showed Ca_V3.2 specifically within NeuroD1+ EECs and ChgA in Ca_V3.2+ epithelial cells. Mechanical (ΔF/F0 1.6 ± 0.6, n = 4) or chemical stimulation with AITC (ΔF/F0 1.7 ± 0.3, n = 4) induced intracellular Ca^{2+} increase. Mechanosensitive responses were inhibited by Ca^{2+} substitution (−96 ± 1% Ca^{2+} free), partly-selective T-type (−71 ± 9% nickel dose and −61 ± 6% mibefradil dose), and P/Q-type (−80 ± 4% ω-agatoxin IVA), but not L-type Ca_V blockers (2 ± 5% nifedipine dose) (n = 3-18, $P < 0.05$, except nifedipine vs control). Ca_V3.2 knockdown blocked mechanosensitive (ΔF/F0 0.5 ± 0.3, n = 4), but not AITC responses (1.5 ± 0.4 AITC, n = 4), suggesting Ca_V3.2 is specifically required for mechanotransduction.

Conclusions: EEC mechanotransduction requires T- and P/Q-, but not L-type Ca_Vs. Ca_V3.2 knockdown specifically blocks mechano, but not chemosensitivity, suggesting that EECs utilize different signaling pathways for chemo and mechanotransduction.

70 | Conditional deletion of leptin receptor in vagal afferent neurons reduces absorption and storage of nutrients from high-fat diet

Kuei-Pin Huang[1]; Amanda Page[2]; Helen Raybould[1]

[1]*School of Veterinary Medicine, University of California Davis, CA, USA;* [2]*Adelaide Medical School, University of Adelaide, SA, Australia*

Background/objectives: Activation of vagal afferent neurons (VAN) by postprandial gastrointestinal signals terminates feeding and facilitates the digestion and absorption of nutrients. Conditional deletion of the leptin receptor from VAN (Nav1.8-Cre/$LepR^{fl/fl}$; $VAN^{\Delta LepR}$) results in loss of cholecystokinin (CCK)-induced satiation and hyperphagia when mice ingest a chow diet. Surprisingly, $VAN^{\Delta LepR}$ mice are resistant to high-fat diet (HF)-induced obesity. We hypothesized that lack of leptin signaling in VAN reduces responses to meal-related signals, which in turn decreases absorption of nutrients and systemic storage of energy.

Methods: CCK-induced c-fos in hindbrain was measured by immunohistochemistry and mechanosensitivity of VAN was studied by electrophysiology in male $VAN^{\Delta LepR}$ and wild type control (WT) fed chow diet. Six-week-old male $VAN^{\Delta LepR}$ and WT were fed with 45% HF for 4 weeks. The metabolic phenotype and food intake were measured by indirect calorimetry, and absorption and storage of carbohydrates were investigated in refed state.

Results: $VAN^{\Delta LepR}$ had decreased CCK-induced c-fos in nucleus of the solitary tract and reduced mechanosensitivity of gastric mucosal vagal afferent, compared with WT, suggesting impaired response of VAN to gastrointestinal signals. After 4 weeks of HF, WT gained more body weight and fat mass compared to $VAN^{\Delta LepR}$, but there was no difference in food intake or energy expenditure. $VAN^{\Delta LepR}$ had reduced expression of Glut2, Glut5 and Sglt1 in jejunum and Sglt1-mediated Na^+ flux was decreased, suggesting decreased absorption of carbohydrate. $VAN^{\Delta LepR}$ had fewer hepatic lipid droplets and decreased expression of enzymes for *de novo* lipogenesis (Acaca and Scd1) and endogenous lipoprotein pathway-associated protein (Apob, Apoe, and Ldlr) in liver compared to WT, suggesting decreased long-term storage of carbohydrate in $VAN^{\Delta LepR}$.

Conclusions: Lack of leptin signaling in VAN reduces responsiveness to gastrointestinal signals and impairs the intestinal absorption of carbohydrates and *de novo* lipogenesis in the liver. This study reveals a compensatory mechanism in VAN to decrease susceptibility to HF-induced obesity.

82 | Mechanisms linking low-calorie sweeteners to impaired glycaemic control

Denise Kreuch[1,2]; Kerry Ivey[3]; Fredrick M. Mobegi[3,4]; Lex Leong[3,4]; Nicole J. Isaacs[1,2]; Nektaria Pezos[1,2]; Michael Horowitz[1,5]; Christopher K. Rayner[1,5]; Geraint B. Rogers[3,4]; Richard L. Young[1,2,5]

[1]*Adelaide Medical School, The University of Adelaide, South Australia;* [2]*Lifespan Nutrition, South Australian Health & Medical Research Institute;* [3]*Precision Medicine, South Australian Health & Medical Research Institute;* [4]*Medicine & Public Health, Flinders University;* [5]*Centre of Research Excellence in Translating Nutritional Science to Good Health, The University of Adelaide*

Background: High consumption of beverages sweetened with low-calorie sweeteners (LCS) is linked to an increased risk of type 2 diabetes (T2D) in humans, but the mechanisms are unknown. LCS activate intestinal sweet taste receptors to trigger gut hormone release, increase intestinal glucose absorption and trigger gut dysbiosis in animals. It unknown whether LCS exerts these effects in humans, and if so, whether postprandial glycaemia is adversely affected.

Methods: 36 healthy subjects (aged 29 ± 2 years, 16 female) were randomised in double-blind manner to supplementation with capsules containing LCS (92 mg sucralose + 52 mg acesulfame-K, N = 19) or placebo (N = 17), taken three times daily over 2 weeks. Subjects underwent non-sedated endoscopy incorporating a 30 minutes intraduodenal glucose infusion (3 kcal/min, including 3 g of the glucose analogue 3-O-methyl glucose, 3-OMG) with biopsy and stool collection, before and after intervention. Glucose absorption (serum 3-OMG), plasma glucose and gut peptides were measured, and incremental areas under the curve (iAUC, over 120 min) compared by

ANCOVA. Microbiome changes were assessed by shotgun sequencing; taxonomic and functional characteristics were determined using MetaPhlAn2 and HUMAnN2 abundance.

Results: LCS augmented glucose absorption (15% $P \leq 0.05$) and glycaemic responses to enteral glucose (26% $P \leq 0.01$) and showed a trend to attenuate release of glucagon-like peptide-1 (GLP-1, 32% $P \leq 0.1$). LCS reduced commensal bacteria from the *Ruminococcaceae* family and increased opportunistic pathogens from *Streptococcaceae* and *Odoribacteraceae* families. Changes in microbiome composition and genetics due to LCS correlated with host changes in blood glucose, 3-OMG and GLP-1, while *Eubacterium rectale* and *Bacteriodetes uniformis* were identified as moderators and mediators of LCS effects, respectively.

Conclusions: Our findings support the concept that dietary LCS supplementation impairs control of postprandial glycaemia in healthy humans by dysregulating glucose uptake and disposal, and secondary to dysbiosis of gut bacteria.

Clinical Trial Registration Number: ACTRN12615000866505
Supported by: NHMRC

89 | Sacral nerve stimulation inhibits the MAPK/NF-κB signaling pathway and promotes Treg-Th1/Th17 cell balance and self-renewal of enteric nervous system in TNBS-induced inflammation in rats

Yan Meng; Jiande D. Z. Chen
Division of Gastroenterology and Hepatology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction: 2,4,6-trinitrobenzene sulfonic acid (TNBS) is known to induce inflammation through triggering the MAPK/NF-κB pathway and activation of T helper cells. Recently, sacral nerve stimulation (SNS) was reported to exert an anti-inflammatory effect on TNBS-induced colitis. The aim of this study was to investigate whether the SNS anti-inflammatory effect was mediated via MAPK/NF-κB signaling pathway and/or balancing Th1/17-Treg cells. Meanwhile, we also explored if SNS could alter self-renewal of neurons in myenteric plexus.

Methods: Forty male Sprague-Dawley (SD) rats were implanted wire electrodes unilaterally at sacral nerve. One week later, the rats were administrated with TNBS intra-rectally. Five days later, 20 of the rats were treated with SNS 1 hour daily for 10 days with the optimized parameters derived from previous studies and the other 20 rats were treated with sham-SNS (exactly the same setting but SNS at 0 mA). Additional 20 rats were treated with intra-rectal injection of saline, serving as controls. Animal behaviors and various inflammatory factors were assessed by the disease activity index (DAI), macroscopic score, microscopic score, fluorescence-activated cell sorter and western blot. Longitudinal muscle myenteric plexus (LMMP) was studied by immunohistochemistry.

Results: (a) Compared with saline group, the TNBS treatment substantially induced inflammation, increased the percentage of Th1 cells ($P = 0.03$), Th17 cells ($P = 0.02$) and Treg cells ($P = 0.04$); it also increased p-ERK/ERK by 3.3 folds ($P < 0.01$) and p-JNK/JNK by 21.7 folds ($P < 0.001$) and increased nuclear translation of NF-κB p65 by 4.5 folds ($P < 0.01$); (b) compared to sham-SNS, SNS significantly decreased DAI (area under the curve: 64.3 ± 3.8 vs 49.5 ± 3.2, $P < 0.01$), macroscopic scores (5.85 ± 0.9 vs 2.55 ± 0.6, $P = 0.03$) and microscopic scores (4.6 ± 1.1 vs 2.7 ± 0.8, $P = 0.04$) and normalized the colon length; (c) in colon tissues, compared with sham-SNS, SNS reduced the percentage of Th1 cells (8.87 ± 2.32% to 5.40 ± 1.39%, $P = 0.04$) and Th17 cells (12.35 ± 1.61% to 9.75 ± 1.17%, $P = 0.04$) but increased Treg cells (15.73 ± 2.81% to 20.15 ± 2.24%, $P = 0.03$); (d) SNS reduced the percentage of the phosphorylation of MAPKs compared to Sham-SNS (p-ERK/ERK: 22.5%, $P = 0.03$; p-JNK/JNK: 25.6%, $P = 0.04$) and prevented nuclear translocation of NF-κB p65 by 40.7% ($P = 0.02$, vs sham-SNS); (e) the percentage of choline acetyltransferase (ChAT) neurons were decreased by TNBS but reversed by SNS (19.06 ± 2.07% to 25.68 ± 3.56%, $P = 0.02$). The percentage of nitric oxide synthase (NOS) neurons was increased by TNBS but decreased by SNS (17.21 ± 1.27% to 13.34 ± 1.63%, $P = 0.03$).

Conclusions: SNS is effective in inhibiting colon inflammation through the inhibition of the MAPK/NF-κB pathway, balancing of Th1/Th17-Treg cells, and also improving the LMMP neuronal self-renewal.

Keywords: Inflammatory bowel disease; sacral nerve stimulation; inflammatory cytokines; MAPK/NF-κB pathway; balancing of Th1/Th17-Treg cells; longitudinal muscle myenteric plexus; neuronal self-renewal.

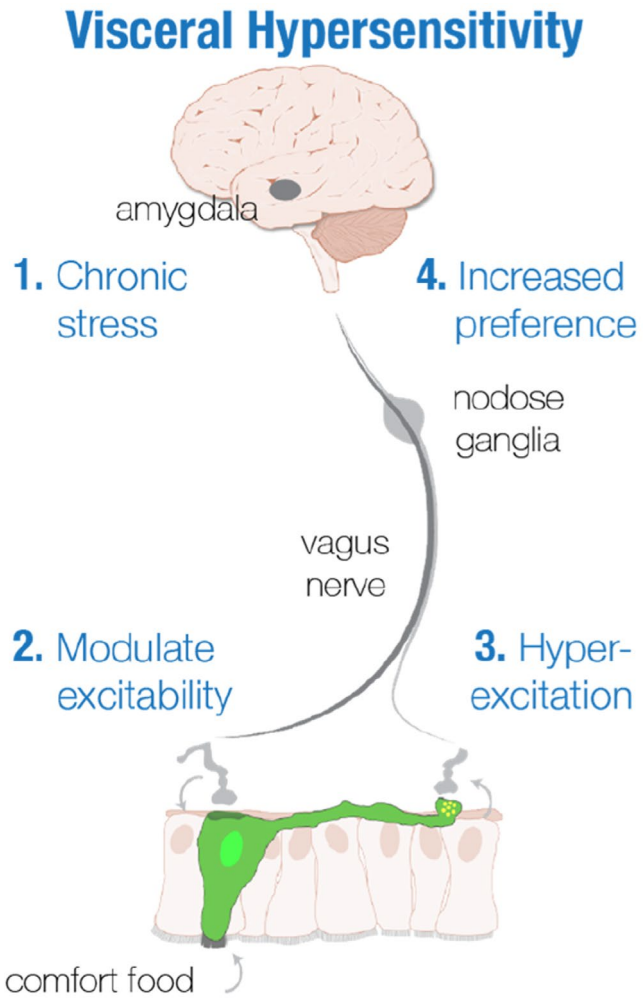
90 | Connecting the brain to the gut: Efferent neurocircuitry of neuropod cells

M. Maya Kaelberer; Marguerita E. Klein; Diego V. Bohórquez
Duke University

Background/Objectives: Chronic physical and/or emotional stress causes several physiological symptoms throughout the body. Chronic stress is associated with an increase in sensitivity and baseline activity in the amygdala — the fear and anxiety center in the brain, that telegraphs stress through neurocircuitry. Largely overlooked consequences of chronic stress are functional gastrointestinal (GI) disorders mostly caused by visceral hypersensitivity. Visceral hypersensitivity is a condition causes the patient's GI tract to overreact to innocuous sensory input, such as bacteria or food. Chronic stress specifically leads to an increased preference in high-fat, high-sugar food, i.e. comfort food. This implies that food perception, or sensory transduction, may be affected by chronic stress. The sensory cells of the gut epithelium are known as enteroendocrine cells. Previously we discovered that a subset of enteroendocrine cells form synaptic connections with vagal neurons. This connection is able to transduce

a sugar stimulus to the brain in milliseconds using glutamate as a neurotransmitter (Kaelberer et al., *Science*, 2018). We call these synaptically connected sensory cells, neuropod cells. The *objective* of this study is to document the neuroepithelial circuit that connects the amygdala neurons to sensory neuropod cells in the small intestine.

Methods: Here we used immunohistochemistry to identify whether sensory epithelial cells in the small intestine have the structural proteins to receive efferent synaptic connections. In addition, we developed a Cre-expressing rabies virus to trace the multi synaptic efferent circuitry from the intestinal lumen to the brain.
Results: We found that 79% ± 4% of Cck-expressing small intestine epithelial cells express the post-synaptic protein, PSD95. Using a multi-synaptic rabies virus, we traced neuropod efferent connectivity to the central amygdala, through the dorsal motor nucleus of the vagus. Showing a direct synaptic circuit that has the potential to modulate sensory transduction at the epithelial layer.
Conclusions: This circuit between the amygdala and neuropod cells of the small intestine is a potential pathway for the brain to modulate gut sensory processing.



105 | LIG3 mutations cause a new type of mitochondrial neurogastrointestinal encephaloneuromyopathy

Francesca Bianco[1]; Christian Bergamini[2]; Valerio Carelli[3]; Georgios Kellaris[4]; Nicholas Katsanis[4]; Marco Seri[1]; Paolo Clavenzani[5]; Roberto De Giorgio[6]; Elena Bonora[1]

[1]*Department of Medical and Surgical Sciences, St. Orsola Hospital, University of Bologna, Italy*; [2]*Department of Pharmacy and Biotechnology, University of Bologna, Italy*; [3]*IRCCS Istituto delle Scienze Neurologiche di Bologna, UOC Clinica Neurologica, Bologna, Italy*; [4]*Center for Human Disease Modeling, Duke University, Durham, USA*; [5]*Department Veterinary Medical Sciences, University of Bologna, Italy*; [6]*Department of Medical Sciences, St. Anna Hospital, University of Ferrara, Italy*

Background & Aims: Chronic intestinal pseudo-obstruction (CIPO) is one of the most severe forms of gastrointestinal (GI) dysmotility characterized by recurrent non-mechanical, sub-occlusive episodes and digestive symptoms. Herein, we showed that genetic analysis unraveled variants causing severe dysmotility in three male siblings born from healthy unrelated parents and presenting with CIPO and neurological impairment (leukoencephalopathy).
Methods: A next-generation sequencing analysis using DNA from study subjects was performed. Morpholino and genome-editing studies in the zebrafish were used to model the observed clinical phenotype based on detected variants. Also, gut tissues and cultured skin fibroblasts from patients carrying the gene variants were investigated.
Results: Sequence analyses of DNA from probands revealed the heterozygous variants p.K537N (paternal) and p.G964R (maternal) in *LIG3*, which encodes the DNA ligase 3 involved in nuclear and mitochondrial DNA (mtDNA) maintenance. *LIG3* mutations affected highly conserved residues causing reduced ligase activity and protein levels. Disruption of *lig3* in the zebrafish reproduced brain alterations and gut dysmotility with markedly delayed transit; this phenotype was rescued by the expression of wild-type *LIG3* mRNA, but not by mRNA encoding the p.K537N and p.G964R variants, supporting a loss of function mechanism in this condition. *LIG3* mutations induced mitochondrial dysfunction, impairing maintenance and repair of mtDNA.
Conclusion: Our study identified a novel mitochondrial syndrome with GI encephaloneuromyopathy due to LIG3 mutations. Further research is needed to determine the exact mechanisms involved in intestinal neuromuscular dysmotility leading to CIPO and in leukoencephalopathy.
Grant Support: Telethon GGP15171 and Fondazione Cassa di Risparmio di Bologna.

120 | Detection of colitis induced intestinal and kidney fibrosis using α-pro-MMP9 immuno-PET

Nicole Dmochowska[1]; William Tieu[2]; Marianne Keller[3]; Courtney Hollis[2]; Melissa Campaniello[1]; Chris Mavrangelos[1]; Prab Takhar[2]; Patrick Hughes[1]

[1]*University of Adelaide and SAHMRI;* [2]*MITRU, SAHMRI;* [3]*PIRL, SAHMRI*

Introduction: Intestinal fibrosis is a common complication of inflammatory bowel disease (IBD) but remains difficult to diagnose and treat. Matrix metalloproteases (MMPs) and tissue inhibitors of matrix metalloproteases (TIMPs) have key roles in fibrosis and are therefore potential targets for detection of fibrosis by immunoPET.

Methods: Mice were administered 2% DSS treated water for 5 days + normal water for 3 days (inflamed), 3 cycles of DSS + 8 days normal water (fibrotic), or untreated (control). Colonic and kidney collagen content, innate cytokine, MMP and TIMP-1 and faecal MPO concentrations were analysed by multiplex/ELSIA. α-pro-MMP-9 $F(ab')_2$ fragments were engineered, conjugated to ^{89}Zirconium (^{89}Zr) and administered i.v. o/n before PET imaging. Bio-distribution was determined ex-vivo by Cherenkov imaging (IVIS spectrum) and gamma-counts.

Results: Colonic collagen concentrations were increased in fibrotic mice ($P < 0.01$, N = 6). Colonic IL-1α, IL-1β, IL-6 and TNF-α concentrations were increased in inflamed mice ($P < 0.05$, N = 6) but did not differ between fibrotic and control mice. MMP-2, -3, -8, pro-MMP-9 and TIMP-1 were increased in inflamed relative to control mice ($P < 0.001$, N = 6). Only pro-MMP-9 remained increased in fibrotic relative to inflamed mice. ^{89}Zr-pro-MMP-9 $F(ab')_2$ uptake was increased in the intestine ($P < 0.01$, N = 5) but also in the kidney ($P < 0.001$) of fibrotic mice. Collagen and pro-MMP-9 concentrations were also increased in the kidney in fibrotic mice ($P < 0.01$, N = 6).

Conclusion: ^{89}Zr-pro-MMP-9 $F(ab')_2$ detects colitis induced intestinal fibrosis and associated kidney fibrosis. This is the first immunoPET study of fibrosis in any tissue, and highlights the utility of coupling antibody specificity to PET sensitivity for detecting disease targets in tissues that are distant to disease origin.

131 | Vagus nerve stimulation ameliorates murine colitis by dampening intestinal inflammation

Nathalie Stakenborg; Elisa Meroni; Pedro J. Gomez-Pinilla; Javier Aguilera-Lizarraga; Morgane Florens; Michelle Stakenborg; Gianluca Matteoli; Guy E. Boeckxstaens

Department of Chronic Diseases, Metabolism and Ageing; Translational Research Center for GastroIntestinal Disorders, University of Leuven, Leuven, Belgium

Background: We previously showed increased susceptibility to dextran sulfate sodium (DSS)-induced colitis in vagotomised mice. Here, we evaluated if vagus nerve stimulation (VNS) is able to reduce DSS colitis and aimed to unravel the mechanisms involved.

Methods: Colitis was induced in wild type mice by 2.5% DSS administration in drinking water for 5 days. VNS (5 Hz, 1 mA, 1 ms for 5 minutes) was applied 1 day prior (d-1) or 4 days (d4) of DSS administration to evaluate changes in mucosal permeability and the inflammatory response, respectively. To assess the intestinal permeability colon tissue of d-1 VNS and sham-stimulated mice were mounted in Ussing chambers. Colonic monocytes, immature and differentiated macrophages were sorted from mice that underwent VNS or sham-stimulation at d4 and gene expression was studied.

Results: VNS applied prior to DSS induction reduced disease activity index (sham 2.8 ± 0.73 vs VNS 0.8 ± 0.58; $P < 0.01$) and improved histology scores (sham: 2.6 ± 0.9 vs VNS: 0.6 ± 0.4; $P < 0.05$). VNS increased epithelial cell proliferation (sham: 226 ± 19 vs VNS: 357 ± 22 Ki67-positive cells/field; $P < 0.01$) and diminished apoptosis (sham: 35 ± 7% vs VNS: 10 ± 2% TUNEL-positive cells; $P < 0.05$), resulting in a tendency to improved intestinal barrier function compared to sham-stimulation. VNS applied at d4 decreased the influx of monocytes (sham: $2.8 \pm 0.2 \times 10^5$ vs VNS: $0.5 \pm 0.1 \times 10^5$ cells,) monocyte-derived macrophages (sham: $2.8 \pm 0.5 \times 10^5$ vs VNS: $1.5 \pm 0.2 \times 10^5$ cells) and neutrophils (sham: $2.7 \pm 0.5 \times 10^5$ vs VNS: $1.7 \pm 0.6 \times 10^5$ cells), and reduced pro-inflammatory cytokine production (i.e. TNFα, CXCL1 and IL6) by immature macrophages compared to sham-stimulation.

Conclusions: VNS reduced DSS-induced colitis by improving epithelial integrity and dampening intestinal inflammation by reducing cytokine expression in immature macrophages. Our data further underscores the potential of VNS as a novel therapeutic approach for inflammatory bowel disease.

138 | Correlation between antroduodenal manometry and histopathology in paediatric intestinal pseudo-obstruction

Atchariya Chanpong[1,2,3]; Osvaldo Borrelli[1,3]; Simon Eaton[3]; Anna Rybak[1]; Efstratios Saliakellis[1]; Keith J. Lindley[1]; Michael Ashworth[4]; Nikhil Thapar[1,3,5]

[1]*Neurogastroenterology & Motility Unit, Department of Paediatric Gastroenterology, Great Ormond Street Hospital for Children NHS Foundation Trust;* [2]*Department of Paediatrics, Faculty of Medicine, Prince of Songkla University;* [3]*Stem Cells and Regenerative Medicine, UCL Great Ormond Street Institute of Child Health;* [4]*Department of Paediatric Histopathology, Great Ormond Street Hospital for Children NHS Foundation Trust;* [5]*Gastroenterology, Hepatology and Liver Transplant, Queensland Children's Hospital*

Background: Currently, the diagnosis of paediatric intestinal pseudo-obstruction (PIPO) is primarily based on clinical picture, which mimics small intestinal obstruction in the absence of luminal occlusion. Antroduodenal manometry (ADM) and histopathology have been applied to definitively diagnose PIPO and classify subtypes of disease (neuropathy, myopathy and neuro-myopathy). However, there

are limited data regarding the reliability of current protocols for ADM analysis as well as how well this correlates with histopathological findings.

Objective: To develop enhanced analysis of gastrointestinal contractile patterns on ADM, including a practical scoring system, and correlate these with histopathology from small intestinal full thickness biopsies in the same children with PIPO.

Methods: We included all patients referred for PIPO investigation to the Gastroenterology Service at Great Ormond Street Hospital between April 2012–July 2019, and who had both ADM (completed study ≥20 hours) and histopathology results available to review.

All ADM tracings were thoroughly analysed using both standard (original ADM) and novel (enhanced ADM) contractile parameters. Using the enhanced analysis, an ADM score based on key contractile elements across the entire study was generated. Both this score and the original ADM analysis were correlated with histopathological findings.

Results: There were 15 PIPO and 11 non-PIPO patients included in this study. Of 15 patients, 14 (84.6%) were reported to have abnormal histopathologic features: 4 neuropathy, 2 neuro-myopathy, 1 myopathy; and 7 patients had changes of uncertain clinical significance. No significant agreement was found between the diagnostic labels from 'original ADM' analyses and histopathology ($\kappa = 0.012$; $P = 0.91$). However, enhanced ADM scores were different between subtypes of PIPO, especially between neuropathic and non-neuropathic groups (24 vs 13; $P = 0.03$); and showed better correlation with histopathology (Figure 1). Median ADM scores were significantly higher in PIPO, compared to non-PIPO patients (14 vs 8; $P < 0.001$).

Conclusions: PIPO diagnostic labels derived from currently applied analyses of ADM tracings do not correlate with abnormalities seen on histopathology. Our scores derived from enhanced ADM analyses show better correlation with histopathology. We propose to further validate the enhanced ADM analysis and scoring for clinical use in patients with presumed PIPO.

159 | Coupling properties of dopamine/ghrelin receptor heteromers that are controllers of gastrointestinal functions

Linda J. Fothergill[1,2,3]; Andrea Fanjul[4]; Sebastian G. B. Furness[3]; Birgitte Holst[5]; Jill Wykosky[4]; Wendy Winchester[4]; Paul Wade[4]; John B. Furness[1,2]

[1]*Florey Institute of Neuroscience and Mental Health, Australia;* [2]*Department of Anatomy and Neuroscience, University of Melbourne, Australia;* [3]*Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Australia;* [4]*Takeda Pharmaceuticals, Gastroenterology Drug Discovery Unit, San Diego, CA, USA;* [5]*Institute for Biomedical Sciences, Copenhagen*

Background and Objectives: Recent studies suggest that heteromeric dopamine (DRD2) and ghrelin (Ghr) receptors are involved in the control of appetite and colorectal function. The presumed natural ligand is dopamine, because ghrelin is absent from regions predicted to express DRD2/GhrR heteromers. We have used transfected cells to study the receptor pharmacology and G-protein coupling when DRD2 and GhrR are expressed together or separately.

Methods: The human and rat receptors were stably expressed in HEK cells. Responses were assayed using Ca^{2+} mobilization or inhibition of forskolin stimulated adenylyl cyclase. Second messengers are being investigated by knock out and replacement.

Results: In cells expressing only DRD2, dopamine and DRD2 agonists inhibited forskolin induced cyclic AMP, but had no effect on Ca^{2+} levels. Ghrelin had no effect. Conversely, in cells expressing only GhrR, ghrelin and the ghrelin receptor agonists, capromorelin and relamorelin, promoted intracellular Ca^{2+} mobilisation, but dopamine had no effect. In DRD2/GhrR cells, both ghrelin receptor agonists and dopamine receptor agonists increased Ca^{2+} signal (EC50 ghrelin, 2.3 nM; dopamine, 1 nM). The GhrR antagonists/ inverse agonists, YIL781 and JMV2959 fully blocked the effects of both ghrelin and dopamine receptor mediated calcium responses. The DRD2 antagonist, raclopride, partially inhibited the GhrR dependent Ca^{2+} response, but fully blocked DRD2 agonism at either pathway.

Conclusions: An apparently reciprocal functional interaction occurs between DRD2 and GhrR when they are expressed together. Dopamine elevates Ca^{2+} when DRD2 is expressed with GhrR, but not when it is expressed alone. This GhrR-dependent effect of dopamine is blocked by GhrR antagonism. We conclude that dopamine binding to DRD2 causes a conformational change in GhrR, causing GhrR to activate a Ca^{2+} signal though G protein coupling to GhrR. There is evidence that coupled DRD2 interferes with ghrelin agonist signalling through GhrR.

161 | Intraluminal nutrients and distension trigger changes in intracellular calcium activity in the enteric nervous system of adult mice

Jean-Baptiste Cavin; Joel Glover; Wallace K. MacNaughton; Keith A. Sharkey

Department of Physiology & Pharmacology, University of Calgary, Calgary, AB, Canada

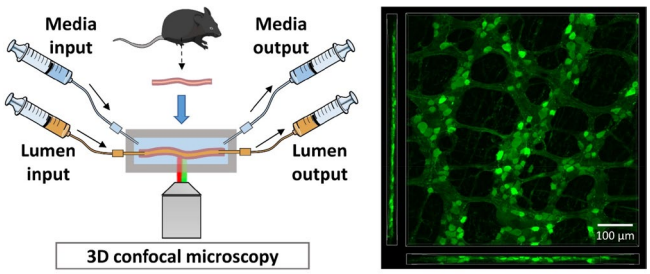
Background/objectives: It has long been hypothesized that enteric neurons are able to detect nutrients present in the intestinal lumen, but direct evidence in intact preparations of the intestine is lacking. Our objective was to measure neuronal activity in the enteric nervous system (ENS) in response to luminal nutrients in the presence or absence of intestinal distension.

Methods: We have designed chambers allowing us to visualize, record and quantify intracellular calcium activity in 3D live-cell confocal recordings of neurons from intact, full-wall thickness segments of mouse intestine. Mice expressing the genetically encoded calcium reporter Gcamp6 under the control of the pan-neuronal Wnt1 promoter were used to record intracellular calcium activity in neurons from the jejunum and colon, while perfusing nutrients (Ensure®) through the lumen. Krebs solution was used as intraluminal control

and distension was generated by gentle inflation with the perfusate. KCl (50 mM bath concentration) served as positive control. 3D videos of calcium fluorescence in the ENS were acquired with a Nikon-A1R confocal microscope and calcium dynamics were analyzed using Imaris (Bitplane).

Results: Perfusion of the intestine with Ensure® reversibly increased the fluorescence intensity in reactive neurons by 670 ± 270% ($(\Delta F/F)$ ± SEM) in the jejunum and 403 ± 62% in the colon. The peak amplitude of the response was measured on average 5-6 minutes after the initiation of the nutrient perfusion. The proportion of reactive neurons was 66 ± 8% and 90 ± 5% in the jejunum and colon, respectively, indicating that subpopulations of neurons might differentially react to luminal stimuli. Distension increased baseline level of fluorescence in the ENS and totally changed how neurons responded to intraluminal nutrients or stimulation with KCl.

Conclusions: We report the existence of slow intracellular calcium changes in enteric neurons that vary depending on the lumen contents and in response to distension. Our novel system will allow a better understanding of integrative control by the ENS.



3D Confocal imaging of the myenteric plexus in the intact colon of a Wnt-1 Gcamp6 mouse. The fluorescence intensity is representative of the calcium concentration in a given cell.

168 | High-resolution colonic manometry reveals postprandial motility differences between constipation subtypes

Victoria Wilkinson-Smith[1]; Lukasz Wiklendt[2]; Maura Corsetti[1]; Luca Marciani[1]; Helen Handford[1]; S. Mark Scott[3]; Robin Spiller[1]; Phil Dinning[2,4]; RECLAIM Study Group

[1]*NIHR Nottingham Biomedical Research Centre, University of Nottingham, UK;* [2]*College of Medicine & Public Health, Flinders University, Australia;* [3]*Neurogastroenterology Group, QMUL, UK;* [4]*Department of Gastroenterology, Flinders Medical Centre, Australia*

Background: Constipation treatments are usually symptom-based rather than targeted at underlying pathophysiology. Although recent MRI studies report reduced colonic motility in functional constipation (FC) but not constipation-predominant irritable bowel syndrome (IBS-C) compared to healthy volunteers (HV), large bowel motility remains poorly defined between these two constipation subtypes. Using high-resolution colonic manometry as the 'gold-standard' our aim was to directly assess distal colonic motility in response to a meal in HV, and in patients with FC or IBS-C.

Methods: 80 participants (37 HV [32F; 34 ± 13 years], 16 FC patients [15F; 46 ± 14 years], and 27 IBS-C patients [26F; 37 ± 13 years]) underwent colonic manometry using a 36-channel solid-state catheter, with sensors spanning the rectum, sigmoid and distal descending colon. Recordings were made 2 hours prior to and after a 700 kCal meal. Significant differences are expressed as the median ratio increase with their 95% credible interval. Using automated analysis, we also compared the post-prandial change in coherence (running correlation between pressure waves in adjacent sensors) between groups.

Results: In the descending and sigmoid colon, but not rectum, meal ingestion induced an increase in the proportion of time with pressure waves of frequency 2-8 cycles per minute (cpm) in all 3 groups. Comparison of this increase between groups showed no difference between IBS-C and HV, but when compared to IBS-C and HV, FC showed a significant 0.71 (0.54-0.93) and 0.66 (0.52-0.84) times decrease, respectively. However, change in post-prandial coherence was significantly reduced in IBS-C compared to both health and FC (See Fig).

Conclusion: Our findings support previous MRI reports of reduced colonic motility in FC but not IBS-C compared to health. Novel analysis of coherence also demonstrates a potential decrease in coordination between pressure waves in adjacent regions in IBS-C, which may underlie their symptoms.

178 | Functional testing in zebrafish to determine causal genes included in large copy number deletions found in Hirschsprung disease patients with additional associated anomalies

Laura E. Kuil[1]; Katherine C. MacKenzie[1]; Clara S. Tang[2]; Maria M. Alves[1]; Alice Brooks[1]; Robert M. W. Hofstra[1,3]; Erwin Brosens[1]; the International HSCR Consortium

[1]*Erasmus University Medical Centre, Rotterdam, The Netherlands;* [2]*University of Hong Kong, Hong Kong, China;* [3]*UCL Great Ormond Street Institute of Child Health, London, UK*

Background & Objective: Hirschsprung disease (HSCR) is characterized by the absence of enteric ganglia, primarily in the distal colon. Approximately 18% of patients have additional anatomical malformations or associated neurodevelopmental disorders. In most of these patients the genetic etiology is unknown and we hypothesize that rare Copy Number Variation (CNV) impacts their disease development.

Methods: We determined Copy Number (CN) changes using SNP-array genotyping arrays and Biodiscovery Nexus CN8.0 (Biodiscovery) in isolated HSCR, patients with associated malformations and patients with a known pathogenic genetic variant. Within

the identified CNVs, candidate genes were selected based on mutation intolerance and expression in the developing mouse ENS.

Results: Rare CN losses were significantly enriched in patients with HSCR and additional anomalies without a known causal variant (n = 23, *P* = 3.64E-7). This was not the case in isolated HSCR (n = 20, *P* = 0.700), or in HSCR patients with a known *RET*, or another causal variant (n = 15, *P* = 0.705). The rare CN losses identified were enriched for variant intolerant genes, expressed in the developing mouse enteric nervous system (*P* = 1.760E-10). Disruption of *ufd1l*, *tbx2* and *akt3* using CRISPR/Cas9 complex injections induced HSCR phenotypes in the injected generation (F0) zebrafish larvae, either by disruption of the gene alone, or in combination with repression of *ret* translation.

Discussion: These data confirms our hypothesis that rare CNV contributes to HSCR with associated anomalies and an unknown genetic etiology. Some of the candidate genes likely also contribute to non-syndromic HSCR patients as *AKT3* as well as *UFD1L* are impacted by CN losses in these patients. In addition, we show that functional genetic testing in zebrafish is a valuable tool to pinpoint the responsible gene(s) in a CNV contributing to the disease.

181 | EphB2 receptor regulates the connectivity and activity of enteric neurons

Raphael Bodin[1]; Vincent Paille[2]; Thibauld Oullier[1]; Tony Durand[1]; Philippe Aubert[1]; Catherine Le Berre-Scoul[1]; Philippe Hulin[3]; Michel Neunlist[1]; Moustapha Cisse[1]
[1]*Université de Nantes, Inserm, TENS, The Enteric Nervous System in Gut and Brain Diseases, IMAD;* [2]*UMR 1280 Physiologie des Adaptations Nutritionnelles, INRA, Université de Nantes, Institut des Maladies de l'Appareil Digestif;* [3]*Plateforme MicroPICell, SFR Santé*

Background/objectives: A highly organized circuit of enteric neurons is required for gastrointestinal functions. However, molecular mechanisms that regulate the connectivity of enteric neurons and their assembly into functional neuronal networks are currently unknown. Hence, a better understanding of mechanisms by which neurotrophic factors regulate enteric neuron circuitry is paramount to understanding the physiology of enteric nervous system. For instance, EphB2, a receptor tyrosine kinase, is essential for neuronal connectivity and plasticity in the brain but so far, its presence and function in the enteric nervous system remains relatively unexplored. Here we examined EphB2 expression in the rat gut and investigated its influence on the connectivity and activity of enteric neurons.

Methods: We used immunohistochemistry and biochemistry on colonic tissues from rat to characterize EphB2 in the enteric nervous system. We dissected EphB2 signaling in primary cultures of enteric nervous system. We examined the impact of EphB2 on the connectivity of enteric neurons in primary cultures. Finally, we determined whether EphB2 regulates the activity of enteric neurons in primary cultures of ENS by an electrophysiology approach.

Results: Here we report that EphB2 is expressed throughout the gut in rats and preferentially by enteric neurons relative to glial cells. Activation of EphB2 with its natural ligand ephrinB2 engages Rac1, a member of the Rac subfamily of Rho-GTPases, and ERK signaling pathways in primary enteric neurons. Conversely, depletion of EphB2 through chronic activation with natural ligand ephrinB2 reduces neuronal plasticity markers such as synapsin I, PSD95 and synaptophysin. Interestingly, EphB2 depletion leads to a reduction of spontaneous miniature synaptic currents of enteric neurons.

Conclusions: Altogether, these findings indicate that EphB2 regulates the connectivity and activity of enteric neurons and identify EphB2 as master regulator of neuronal plasticity in the gut. Thus, EphB2 might represent a novel therapeutic target for pathologies associated with defects in enteric neuronal connectivity.

184 | Inhibition of axonal outgrowth by Sema3A in enteric neurons and its potential implication in Hirschsprung disease

Jacques Gonzales; Catherine Le Berre-Scoul; Anne Dariel; Paul Bréhéret; Michel Neunlist; Hélène Boudin
INSERM UMR 1235 – TENS; IMAD, University of Nantes, France

Objectives: Gut functions are controlled by the enteric nervous system (ENS), a complex network of enteric neurons located throughout the wall of the gastro-intestinal tract. Defects in the formation of neuronal connectivity during development are thought to result in motility disorders but the underlying mechanisms are poorly understood. Here, we studied the expression and the role of Semaphorin 3A (Sema3A) and its receptor neuropilin 1 (NRP1) in ENS development. We also analyzed the expression of Sema3A, Nrp1 and key neuronal molecules in patients with Hirschsprung disease, for which Sema3A mutations have been described.

Methods: Gene and protein expression of Sema3A and NRP1 were analyzed in rat colon throughout postnatal development. Double immunolabelings were performed in whole mount colon for Sema3A or NRP1 with specific markers of glia (S100β), neurons (Hu, Tuj-1), and muscle cells (αSMA). The impact of Sema3A on neuronal outgrowth was assessed in rat gut explants and cultures of enteric neurons. Finally, post-operative bowel segments from control subjects and HSCR patients were analyzed for Sema3A, NRP1, and synapsin 1 expression.

Results: A peak of mRNA expression for Sema3A and Nrp1 was observed in distal colon at 7 days, corresponding to a stage of intense neural circuit remodeling. Sema3A immunoreactivity exhibited a punctate immunoreactivity in ganglia, a pattern consistent for a secreted protein. Nrp1 was found in neurons, mainly associated with axons. Enteric neurons from gut explants or in primary cultures showed a strong reduction in axonal outgrowth when cultured in the presence of Sema3A. Analyses of resected ganglionic segment from HSCR patients indicated a reduced expression of synapsin 1, a key molecule of neuronal connectivity. Moreover, synapsin 1 expression was inversely correlated with Sema3A protein expression.

Conclusion: This study provides evidence that Sema3A restrains axonal outgrowth during ENS development. In HSCR, alterations of ENS connectivity, possibly involving Sema3A, could have marked consequences on gut functions.

191 | Anti-Inflammatory actions of an intraluminally acting 5-HT4 receptor agonist

Brigitte Lavoie[1]; Melody M. Haag[1]; Molly C. Hurd[1]; Grant W. Hennig[1]; John Parkinson[2]; Jill Wykosky[2]; Gary M. Mawe[1]
[1]*Department of Neurological Sciences, University of Vermont, Burlington, VT, USA;* [2]*Takeda Pharmaceuticals, San Diego, CA, USA*

Objective: The 5-HT4 receptor is expressed by most epithelial cells in the colon, and luminal administration of 5-HT4 agonists promote motility (PMID 22226658) and attenuate colitis (PMID 27480173). This investigation was conducted to test whether intraluminally acting 5-HT4 agonists exert a protective effect in mouse models of colitis.
Methods: Models studied included IL-10 knockout, DSS, and TNBS colitis. Mice were treated daily for three weeks, by enema, with an intraluminally acting 5-HT4 agonist (ILA-5-HT4 agonist; 10 mg/kg), the agonist plus the 5-HT4 antagonist (GR-113808; 1 mg/kg), or vehicle. Colitis was evaluated using disease activity index (DAI) and blinded histological damage scores. Effects of the agonist on cell migration using the Caco-2 wound healing assay were also evaluated.
Results: Mice receiving the ILA-5-HT4 agonist had significantly lower cumulative and daily DAI scores in all colitis models compared to vehicle-treated mice ($P \leq 0.01$). Agonist plus antagonist treatment was tested in the IL-10 KO model where the antagonist blocked the protective effect of the agonist, with the DAIs of these mice being comparable to those of vehicle-treated mice. Treatment with the ILA-5-HT4 agonist reduced histological damage scores in the IL-10 KO mice ($P \leq 0.01$) in an antagonist-sensitive manner. Scratch closure in monolayer cultures of Caco-2 cells was significantly faster in preparations treated with the ILA-5-HT4 agonist vs vehicle, whereas no difference between vehicle and agonist plus antagonist was observed.
Conclusions: These findings demonstrate that administration of a 5-HT4 agonist that acts intraluminally reduces the development of inflammation in three different models of colitis. These findings support the concept that mucosal 5-HT4 receptors could represent a novel therapeutic target for the treatment of colitis.

192 | Different luminal nutrients activate distinct patterns of myenteric neurons

Candice Fung[1]; Marlene Hao[2]; Jan Tack[1]; Werend Boesmans[3]; Pieter Vanden Berghe[1]
[1]*University of Leuven, Leuven, Belgium;* [2]*University of Melbourne, Parkville, Australia;* [3]*Hasselt University, Hasselt, Belgium*

Background: Monitoring of ingested nutrients by an organism is essential for balancing energy input. The gastrointestinal tract plays an important role in this homeostasis. Nutrient signals sensed by specialized enteroendocrine cells in the epithelium are conveyed to the enteric nervous system (ENS) to initiate intestinal reflexes facilitating digestion and absorption. However, the extent to which the ENS is 'aware' of the luminal composition remains elusive. We addressed whether there are specific enteric pathways dedicated to detecting different luminal nutrients.
Methods: Calcium imaging was performed on intact jejunal preparations from Wnt1-cre; R26R-GCaMP3 mice, which express the fluorescent calcium indicator GCaMP3 in their ENS. Glucose (300 mM), acetate (100 mM), and L-phenylalanine (100 mM), as a model sugar, short chain fatty acid, and amino acid respectively, were perfused onto the mucosa whilst imaging the underlying enteric neurons. Nutrient transport or diffusion across the mucosa was mimicked by pressure ejecting nutrients from a micropipette impaled through the epithelium of a villus to target the containing nerve endings, or by applying nutrients onto ganglia in peeled preparations. Responders were further classified by their cell size and neurochemistry using *post-hoc* immunolabeling.
Results: Glucose, acetate, and L-phenylalanine perfused onto the mucosa each evoked Ca^{2+} transients in distinct subsets of myenteric (17 ± 6%, 12 ± 2%, and 9 ± 2%) and submucosal neurons (21 ± 4%; 24 ± 7%, and 23 ± 3% of total neurons within the field of view, respectively). The cell size ($P < 0.0001$; One-way ANOVA) and proportions of calbindin$^+$ and nNOS$^+$ myenteric neurons that responded differed significantly between the nutrients ($P < 0.0001$; $\chi 2$ test), while submucosal responders were predominantly cholinergic (98 ± 2% of total responders) and of similar size. Nutrients applied into villi or onto ganglia did not elicit neuronal responses, indicating that nutrients are first sensed at the epithelium.
Conclusions: Different nutrients applied to the epithelium triggered distinct patterns of myenteric neuronal activation, suggesting that the ENS is able to discriminate between different compositions of luminal content such that it can act accordingly.

212 | Nitric oxide modulates intracellular Ca^{2+} in intramuscular interstitial cells of Cajal of the mouse internal anal sphincter

Karen I. Hannigan; Kathleen D. Keef; Caroline A. Cobine
Department of Physiology and Cell Biology, Reno School of Medicine, University of Nevada, Reno, NV, USA

Background/Objectives: Nitric oxide (NO) is an important inhibitory neurotransmitter in the internal anal sphincter (IAS). Intramuscular

interstitial cells of Cajal (ICC-IM) are electrically coupled to smooth muscle cells (SMCs) and mediate nitrergic neuromuscular transmission (NMT) in other gastrointestinal (GI) regions. Nitrergic inhibitory junction potentials (IJPs) in the IAS persist in the presence of the voltage-dependent Ca^{2+} channel (VDCC) blocker nifedipine indicating that these events occur independently of VDCCs. We have previously characterized ICC-IM in the Kit-GCaMP6f mouse IAS and have found two functionally distinct subtypes. Type I ICC-IM display activity similar to ICC-IM in other GI regions, i.e., asynchronous localized Ca^{2+} release events that are insensitive to nifedipine. In contrast, Type II ICC-IM generate VDCC-dependent synchronized whole cell Ca^{2+} transients occurring at the slow wave frequency. The present study examined how nitrergic NMT in the IAS modifies Ca^{2+} transients in Type I and Type II ICC-IM.

Methods: Confocal spinning disc microscopy was used to image Ca^{2+} transients in the IAS of mice that express GCaMP6f in ICC (Kit-GCaMP6f). Preparations were placed submucosal surface upwards and imaged at 20x. Inhibitory motor neurons were stimulated with electrical field stimulation (EFS; 5 Hz, 10 seconds) in the presence of atropine (1 μM), guanethidine (1 μM) and MRS2500 (1 μM).

Results: Nitrergic NMT inhibited Ca^{2+} transients in both Type I and Type II ICC-IM. In Type I ICC-IM Ca^{2+} transients were inhibited in a VDCC-independent manner, i.e., via inhibition of Ca^{2+} release from the endoplasmic reticulum. In Type II ICC-IM, nitrergic NMT abolished VDCC-dependent whole cell Ca^{2+} transients.

Conclusions: The present study demonstrates that nitrergic NMT inhibits Ca^{2+} transients in Type I and Type II ICC-IM. Since both nitrergic IJPs and the NO-mediated inhibition of Ca^{2+} transients in Type I ICC-IM occur independently of VDCC it provides further support for Type I ICC-IM as primary mediators of nitrergic NMT.

Funding: NIH DK078736.

213 | Evaluation of defecation disorders in children with autism spectrum disorder by colonic manometry

Alexander Coe[1]; Jacob Ciricillo[2]; Khalil El-Chammas[3]; Neha Santucci[3]; Alisara Damrongmanee[3,4]; Lin Fei[5]; Chunyan Liu[5]; Ajay Kaul[3]

[1]*Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA;* [2]*University of Toledo College of Medicine, Toledo, OH, USA;* [3]*Department of Gastroenterology and Hepatology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA;* [4]*Division of Pediatric Gastroenterology and Hepatology, Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand;* [5]*Department of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*

Gastrointestinal complaints have been described in 70% of individuals with Autism Spectrum Disorder (ASD). Preclinical data demonstrates delays in gastrointestinal transit in animal models with autism. This study objective was to determine if colonic dysmotility evaluated by colonic manometry (CM), contributed to the defecation disorders in ASD patients. This was a retrospective, propensity-matched, case-control study, with patients with ASD, and control patients without ASD, that had defecatory disorders and underwent evaluation by CM according to published guidelines. Abnormal CM was defined as any study that demonstrated inadequate propagation or low amplitude of colonic contractions, absent colonic contractions or hyperactive contractile patterns. The propensity score (PS) model included the variables: age, race, gender, duration of symptoms, disease severity, history of gastrointestinal surgery, history of gastrointestinal co-morbidity, history of neuro-psychiatric comorbidity and selected interactions. Each ASD patient was matched to a control within a caliper of 10% standard deviation of PS. The CM results of the ASD cohort were compared against non-ASD controls both before and after matching, using a Chi-square test. Thirty-three with ASD and one hundred forty-nine were included. Demographics between both groups differed prior to PS-matching. The cohort with ASD was older as a group, and had marked differences in sex (78.8% male) and race (90.9% white) when compared to the control There was no significant difference in abnormal CM findings between both groups. Abnormal CM was noted in 35 patients among the 149 controls and 10 among the 33 cases (23% vs 30%, $P = 0.41$). After PS matching, 29 patients with ASD and 29 matched controls were compared; there was no significant difference between both groups in abnormal CM findings (9 out of 29 matched controls and 8 out of 29 ASD patients (31% vs 28%, $P = 0.77$). Despite the evidence for delayed gastrointestinal transit in animal models of ASD, there did not appear to be any specific findings on CM that was unique to the ASD cohort. This finding has important implications on the management strategies used to treat disorders of defecation, especially constipation, in children with ASD.

233 | Evaluating the impact of position, volume and consistency on high resolution oesophageal manometry outcomes

Chamara Basnayake; Ans Pauwels; Annelies Geeraerts; Hannelore Geysen; Lien Timmermans; Sawangpong Jandee; Tim Vanuytsel; Nathalie Rommel; Jan Tack

Katholieke Universiteit Leuven, Belgium

Background: The Chicago Classification utilises ten 5 mL liquid swallows in a supine position as the standard high resolution oesophageal manometry (HRM) protocol. To reflect physiological swallows HRM can be performed with varying volumes and consistencies and in an upright position. We aimed to determine the impact on HRM results by 1) the position 2) swallows of differing volume and consistency.

Methods: HRM was performed in healthy volunteers with the following protocol of swallows: Liquids: 10 × 5 mL, 5 × 10 mL, 3 × 10 mL multiple-rapid swallows; Applesauce 5 × 5 mL, 5 × 10 mL; Bread 5 × 2 cm × 2 cm and 5 × 4 cm × 4 cm. All volunteers performed the protocol in three positions: supine, "semi-upright"(sitting 70 degrees in a bed) and "upright"(sitting in a chair). Volunteers performed the study in the supine position first and then were randomised to

perform swallows in "semi-upright" or "upright" position, followed by the position not completed. This analysis includes: Distal Contractile Integral (DCI) and Integrated relaxation pressure-4 (IRP4) results.

Results: Demographics: 32 volunteers, median age 27.7 years, 65.6% female. DCI and IRP4 of 5 mL liquid swallows were not significantly altered by the randomised order that the positions were performed. Position: Median DCI and IRP4 of 5 mL liquid swallows significantly differed (all $P < 0.00001$) between position performed (DCI: 1455 vs 1014 vs 863 and IRP4: 21.6 vs 16.1 vs 15.05, for supine, semi-upright, upright respectively). Volume: Median DCI and IRP4 for liquid 5 mL vs 10 mL swallows differed in the supine (DCI: 1455 vs 1607, $P = 0.09$; IRP4: 21.5 vs 20.3, $P = 0.03$), semi-upright (DCI: 1013 vs 1371, $P = 0.002$; IRP4: 16.1 vs 13.9, $P < 0.00001$) and upright position (DCI: 863 vs 1240, $P = 0.001$; IRP4: 15 vs 11, $P < 0.00001$). Consistency: DCI and IRP4 of supine 5 mL liquid swallows differed to 5 mL applesauce (DCI: 1455 vs 1292, $P = 0.005$; IRP4: 21.6 vs 19.9, $P = 0.06$) and 2 cm × 2 cm of bread (DCI: 1455 vs 2091, $P = 0.01$; IRP4: 21.6 vs 26.3, $P = 0.00001$).

Conclusion: The volume and consistency of a swallow and the position it is performed in significantly alter HRM metrics. As such, different normative values should be used if the Chicago Classification is applied.

Median HRM Metrics by Position and Type of Bolus

	DCI (medians)			IRP4 (medians)		
	Supine	Semi-upright	Upright	Supine	Semi-upright	Upright
5ml liquid	1455	1014*	863*	21.6*	16.1*	15*
10ml liquid	1607	1371	1240*	20.3*	13.9*	11*
5ml apple sauce	1292*	899*	851.5*	19.9	14.4*	11*
10ml apple sauce	1693	1183*	1117*	15.3*	10.1*	7.3*
2x2cm bread	2091*	1321*	971*	26.3*	19.6	13.9*
4x4cm bread	2890.5*	2186*	1759.5	23.1*	17.7*	11.3*

*p<0.05 compared with 5ml liquid in supine position

236 | Pyloric diameter and distensibility responses utilizing EndoFLIP to compare double vs single myotomy in gastroparesis patients following G-POEM

Lydia Watts; Jason Baker; Allen Lee; Kimberly Harer; Nicole Bowers; Ryan Law; William Hasler
Michigan Medicine, Ann Arbor, MI, USA

Objectives: G-POEM (Gastric Per-Oral Endoscopic Myotomy) is emerging therapy for gastroparesis. G-POEM traditionally involves making a single pyloric myotomy via a submucosal tunnel, but double myotomy techniques are proposed to produce improved responses. EndoFLIP measures pyloric function changes after G-POEM. We hypothesized that a double myotomy elicits greater pyloric diameter and distensibility responses to EndoFLIP balloon inflation vs single myotomy.

Methods: G-POEM was performed on 24 gastroparesis patients—14 with one myotomy, 10 with double myotomy. Average increases in mean, maximum, and minimum pyloric diameter to 40 mL balloon inflation and mean distensibility to 50 mL inflation after G-POEM compared to baseline were calculated for the middle 30 seconds of EndoFLIP recordings.

Results: Single myotomy increased mean pyloric diameter from 14.1 ± 2.1 to 15.3 ± 2.1 mm during 40 mL EndoFLIP inflation; double myotomy increased mean diameter from 13.7 ± 1.8 to 16.6 ± 2.9 mm. Mean diameter increases over baseline were greater for double vs single myotomy ($P = 0.03$) (Table). Baseline pyloric diameters exhibited significant variability from a minimum of 12.5 ± 1.7 to a maximum of 16.0 ± 3.1 mm. Maximum diameter increases compared to baseline were greater after double vs single myotomy ($P = 0.02$); minimum diameter increases after G-POEM were similar for single vs double myotomy ($P = 0.27$) (Table). Single myotomy increased mean distensibility from 4.7 ± 2.0 to 5.7 ± 1.5 mm^2/mm Hg during 50 mL EndoFLIP inflation; double myotomy increased mean distensibility from 5.7 ± 2.8 to 9.2 ± 5.4 mm^2/mm Hg. Mean distensibility increases over baseline were greater for double vs single myotomy ($P = 0.05$) (Table).

Conclusions: Double myotomy G-POEMs produce greater increases in mean pyloric diameter and distensibility over baseline in gastroparesis than single myotomy. The greater increases in maximum diameter after double myotomy may reflect enhanced maximal pyloric opening with the additional myotomy. Conversely, similar minimum diameter responses to double and single myotomy may indicate the additional myotomy does not impair pyloric closure. Further studies will define if enhanced responses to double myotomy translate into better gastric emptying and symptom responses after G-POEM.

Table 1 Pyloric responses to G-POEM: comparisons of single vs double myotomy techniques

EndoFLIP response after G-POEM compared to baseline values	Single myotomy	Double myotomy	*P* value
Increase in mean diameter (mm) to 40 mL inflation	1.2 ± 1.7	2.8 ± 1.8	0.03
Increase in maximum diameter (mm) to 40 mL inflation	0.6 ± 3.2	3.6 ± 2.8	0.02
Increase in minimum diameter (mm) to 40 mL inflation	0.3 ± 2.7	1.6 ± 2.5	0.27
Increase in mean distensibility (mm^2/mmHg) to 50 mL inflation	0.9 ± 1.4	3.4 ± 4.2	0.05

243 | An insulin-like peptide 5 related agonist, INSL5-A13, stimulates colorectal propulsion via the relaxin family peptide 4 receptor

John B. Furness; Shanti Diwakarla; Ross A. D. Bathgate; Xiaozhou Zhang; Mohammed Akhter Hossain
Florey Institute, University Of Melbourne, Parkville, Victoria, Australia

Background and Objectives: Insulin-like peptide 5 (INSL5) is a peptide hormone stored in colonic enteroendocrine cells. It acts at the relaxin family peptide 4, RXFP4, receptor that is expressed by enteric neurons in the colon. Similar to insulin and relaxin, INSL5 consists of A and B peptide chains linked by three disulfide bonds, two between the chains and one intrinsic to the A chain. Because of its complex structure, it is difficult to synthesize and to prepare peptide analogues to investigate its roles. Our objective was to develop a peptidomimetic of INSL5 suitable for in vivo investigations, and to determine whether the INSL5/ RXFP4 system is involved in control of colorectal motility.
Methods: We used a rational strategy to design an INSL5-peptidomimetic, INSL5-A13. Solid-phase synthesis was used to make the peptide. Cells were transfected with mouse RXFP4 and used to test potency. The bead expulsion test was used in mice to investigate colokinetic effects of INSL5-A13.
Results: INSL5-A13 was a full agonist at the mouse RXFP4 receptor expressed in HEK cells, with an EC50 of ~9 nM. In mice in which colorectal propulsion was slowed by loperamide (1 mg/kg), INSL5-A13 caused an acceleration of colorectal bead propulsion in the dose range 0.2 to 60 μg/kg, with an EC50 of ~6 μg/kg in vivo. It also accelerated bead propulsion in untreated mice. Colorectal propulsion was about twice as slow in RXFP4-/- mice, compared to their wild-type littermates. Because of the already slowed propulsion, a lower dose of loperamide (0.5 mg/kg) was used. In wild-type, the inhibition of bead expulsion caused by loperamide was reversed by INSL5-A13 (20 μg/kg). Loperamide delayed bead expulsion in RXFP4−/− mice, but INSL5-A13 was ineffective.
Conclusions: It is likely that the INSL5/ RXFP4 system functions, physiologically, to augment colorectal propulsion. Our data suggest that RXFP4 agonists could be useful in the treatment of constipation.

244 | Defecatory function testing of constipated patients with a simulated stool

Hans Gregersen; Ssu-Chi Chen; Cherry Wong; Wing-wah Leung; Tony Mak; Simon Ng; Kaori Futaba
The Chinese University of Hong Kong

Background/objectives: Chronic constipation is a common problem. It may be due to obstructive defecation or slow transit. Patients not responsive to laxatives may be further assessed by transit study, defecating proctogram, anorectal manometry (ARM), balloon expulsion test (BET). However, results often correlate poorly with symptoms. Recently we developed a simulated stool named Fecobionics that integrates several tests and assesses pressures in axial direction during defecation as well as orientation and bending during evacuation (CGH 2018; 16: 981–3). The aim was to study defecatory properties in patients with chronic constipation.
Methods: Nineteen patients with obstructive defecation (15F/4M, 59.5 ± 3.3 years) and 9 patients with slow transit constipation (confirmed with transit study, (6F/3M, 50.6 ± 4.7 years) were enrolled and assessed with Fecobionics, ARM, and BET.
Results: The constipation score was 12.4 ± 0.7 and 14.3 ± 1.5 in obstructed defecators and slow transit patients. Four patients with obstructive defecation could not expel Fecobionics. All patients with slow transit constipation expelled Fecobionics. Nine patients with obstructive defecation and one patient with slow transit did not expel BET. The urge volume for Fecobionics was 72.6 ± 2.7 mL and 61.7 ± 4.7 mL in obstructed defecators and slow transit patients ($P > 0.5$). The urge volume for BET was 104.5 ± 8.9 and 92.7 ± 13.1 ml in obstructed defecators and slow transit patients ($P > 0.5$). The urge volume for Fecobionics was lower than that for BET ($P < 0.005$). The expulsion duration for Fecobionics was 83.6 ± 26.0 and 13.0 ± 6.5 seconds in obstructed defecators and slow transit patients ($P < 0.01$). The expulsion duration for BET was 160.8 ± 30.8 and 96.2 ± 34.5 seconds in obstructed defecators and slow transit patients ($P > 0.05$). The pressure signatures obtained with Fecobionics largely differed between obstructed defecators and slow transit patients. For the subgroup of patients who was capable of expelling the devices, Fecobionics showed higher correlation with the symptom score than BET did ($R^2 = 0.21$ vs $R^2 = 0.12$).
Conclusion: Fecobionics obtained reliable data in assessment of chronic constipation patients. Distinct differences were found between obstructed defecators and slow transit patients. Fecobionics showed closest association with the symptom score. RCG grant #14106717.

264 | Clinical impact of rectal hyposensitivity: A cross-sectional study of 2876 patients with functional constipation

Rebecca E. Burgell[1,2,*]; Paul F. Vollebregt[1,*]; Richard L. Hooper[1]; Charles H. Knowles[1]; S. Mark Scott[1]
[1]*National Bowel Research Centre and GI Physiology Unit, Queen Mary University London, UK;* [2]*Department of Gastroenterology, Alfred Health and Monash University, Melbourne, Vic, Australia*

Background: Normal bowel function requires intact sensory pathways. Diminished rectal sensation (rectal hyposensitivity: RH), is associated with hindgut dysfunction although the clinical impact of this physiological finding remains unclear. This study evaluated the impact of RH on symptoms and allied investigations in patients with constipation.

Methods: Consecutive patients (aged 18-80) attending the Royal London Hospital Gastrointestinal Physiology Unit (2004-2016) for investigation of chronic constipation (core Rome IV criteria positive) were included. Patients completed a comprehensive clinical questionnaire incorporating the Cleveland Clinic constipation score (CCCS) and St Marks incontinence score (SMIS) and underwent anorectal physiology investigations including rectal sensory testing (balloon distension), defecography, and whole-gut transit study (radio-opaque markers).

Results: Of 2876 patients meeting inclusion criteria, 722 (25%) had one or more elevated sensory thresholds (i.e. RH). Of these, 54% had isolated constipation and 46% had co-existent constipation and faecal incontinence. A linear relationship existed between number of elevated sensory thresholds and constipation severity (OR [95%CI] effect per threshold: 0.69 [0.48-0.90]; $P < 0.001$). Increased incidence of specific symptoms was associated with RH including: infrequency of defecation, unsuccessful evacuation, painful evacuation, prolonged toileting, digitation and duration of symptoms >5 years (OR per threshold: 1.29 [1.17-1.42], 1.13 [1.03–1.24], 1.15 [1.05–1.27], 1.14 [1.05–1.24], 1.18 [1.08–1.30] & 1.12 [1.02–1.22], respectively). A functional evacuation disorder was also associated with a higher number of abnormal sensory thresholds (OR per threshold: 1.37 [1.25-1.50], $P < 0.001$) as was megarectum (OR per threshold: 2.52 [2.08-3.05], $P < 0.001$). There was no association with delayed whole gut transit.

Conclusion: Rectal hyposensitivity occurs in a quarter of patients with constipation. Higher number of elevated sensory thresholds is associated with a more severe constipation phenotype. These data, in the largest study to date, provide for the first time, convincing evidence to show that rectal hyposensitivity is a major pathophysiological mechanism in constipation, with recognised clinical impact.

267 | Neonatal exposure to the antibiotic vancomycin induces long term effects on the microbiota, enteric nervous system and host metabolism

Jaime P. P. Foong[1]; Lin Y. Hung[1]; Sabrina Poon[1]; Qinglong Wu[2]; Pavitha Parathan[1]; Anthony Haag[2]; Ruth Ann Luna[2]; Matthew J. Watt[1]; Tor C. Savidge[2]; Joel C. Bornstein[1]

[1]*University of Melbourne, Parkville, Vic, Australia;* [2]*Baylor College of Medicine, Houston, TX, USA*

Background/Objectives: Early life antibiotic exposure is reported to increase susceptibility to diseases later in life including gastrointestinal and metabolic disorders, but little is known about antibiotic-induced disease mechanisms. Vancomycin is given as a prophylactic treatment to pre-term and low birth weight infants. We conducted the first study demonstrating that neonatal exposure to vancomycin disrupts the developing microbiota and nervous system of the gut (enteric nervous system, ENS), and reduces body weights in mouse pups. Here we examined the long-term effects of neonatal vancomycin treatment.

Methods: Mice were given a single daily oral dose of vancomycin or water from birth (postnatal day, P0) to P10 and then left to grow up with standard animal husbandry procedures. At 6-weeks of age, mice underwent MiniSpec NMR analysis of body composition and whole-body metabolic assessment using indirect calorimetry. Mice were then culled, and their colons were removed for 16S rRNA microbiota analysis, measurements of mucosal serotonin by mass spectrometry and examination of enteric neurons and enteroendocrine cells via immunohistochemistry.

Results: Six-week-old mice given neonatal vancomycin had significantly lower microbiota diversity and perturbed microbiota composition. They had decreased faecal dry weight ($P < 0.01$), not seen at P10. They sustained an increased proportion of calbindin+ enteric neurons ($P < 0.05$), decreased number of serotonin+ cells ($P < 0.05$), and reduced levels of the serotonin metabolite, 5-HIAA ($P < 0.001$) in the intestinal mucosa. Neonatal exposure to vancomycin did not affect food consumption or energy expenditure. However, mice given neonatal vancomycin tended to weigh less ($P = 0.08$), had a significantly higher fat mass ($P < 0.001$) and higher expression of glucagon-like peptide 1 (GLP-1) + cells in the intestinal mucosa ($P < 0.05$).

Conclusions: Neonatal exposure to antibiotics induced long-lasting effects on microbiota, ENS and body fat via a mechanism involving mucosal serotonin and GLP-1.

283 | Antibiotic exposure during adolescence disrupts the neurochemistry and function of enteric neurons

Lin Y. Hung[1]; Prapaporn Boonma[2,3]; Pavitha Parathan[1]; Anthony Haag[2]; Ruth Ann Luna[2]; Tor Savidge[2]; Joel Bornstein[1]; Jaime P. P. Foong[1]

[1]*University of Melbourne, Parkville, Vic., Australia;* [2]*Baylor College of Medicine, Houston, TX, USA;* [3]*Faculty of Medicine, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand*

Background/Objectives: The microbial symbionts (microbiota) can influence the enteric nervous system (ENS) within the gut. Although maturation of the ENS and microbiota occur concurrently, the role of microbiota in ENS development remains unclear. Adolescence is a critical developmental period when mouse pups undergo significant changes in diet, behaviour, physiology, and importantly, gut microbiota and ENS. Here, we examine effects of antibiotic exposure during adolescence on microbiota, ENS and gut function.

Methods: We used Wnt1-GCaMP3 mice, which express a genetically encoded Ca^{2+}-indicator in all enteric neurons and glia. Mice were given water or vancomycin (0.5 g/L) in their drinking bottles for 3 weeks during adolescence (from 3-weeks-old) or adulthood (from 8-weeks-old), then sacrificed and had their colons examined. Mucosa and fecal samples underwent 16S rRNA microbiota analysis and measurements of serotonin by mass spectrometry. Structure

and function of submucosal and myenteric neurons were analyzed via immunohistochemistry and calcium imaging. Colonic motility patterns were examined using video imaging.

Results: Vancomycin altered microbial diversity and composition. Vancomycin exposure during adolescence decreased the speed of colonic migrating complexes ($P < 0.05$), but exposure during adulthood had no effect on colonic motility. Vancomycin exposure during adolescence had varying effects on enteric neurochemistry. The proportions of cholinergic (ChAT+) and calbindin+ myenteric neurons were significantly decreased ($P < 0.05$). ChAT+ submucosal neurons were significantly increased ($P < 0.05$). Neuronal nitric oxide synthase+ neurons were unaffected in the myenteric plexus but significantly decreased in the submucous plexus ($P < 0.05$). Proportions of neurofilament M (NFM+) neurons in both enteric plexi were significantly decreased ($P < 0.01$). There was a significant increase in the number of enteric neurons responding to electrical stimulation of interganglionic fibre tracts ($P < 0.0001$). Levels of serotonin in colonic samples were unaffected.

Conclusion: Vancomycin exposure during adolescence has significant effects on the developing microbiota and ENS via a mucosal serotonin-independent mechanism.

287 | Self-reported non-celiac wheat sensitivity (NCWS) in patients with chronic unexplained (functional) gastrointestinal symptoms

Ayesha Shah[1,2,3]; Nicholas Talley[4]; Seungha Kang[1,3,5]; Anh Do[1,2]; Marjorie Walker[4]; Natasha Koloski[1,2,3,4]; Michael Jones[6]; Simon Keely[4]; Mark Morrison[1,3,5]; Gerald Holtmann[1,2,3]

[1]*Princess Alexandra Hospital*; [2]*Translational Research Institute*; [3]*Faculty of Medicine, University of Queensland, QLD, Australia*; [4]*Faculty of Health and Medicine, University of Newcastle, NSW, Australia*; [5]*University of Queensland, Diamantina Institute, Australia*; [6]*Macquarie University, NSW, Australia*

Background & Aims: Alterations of gastrointestinal (GI) sensory, motor or immune function, changes of the gastrointestinal mucosa-associated microbiome (MAM) and psychological comorbidities occur in patients with functional gastrointestinal disorders (FGIDs), and many report NCWS. We hypothesized NCWS related symptoms are linked to an activation of gut homing immune cells in FGID.

Methods: In forty patients with unexplained functional symptoms and 20 asymptomatic control patients, after a negative diagnostic work-up, validated instruments were used to assess GI, extraintestinal symptoms and psychological comorbidities. Symptoms triggered by ingestion of wheat products (NCWS) were assessed by structured interview. Gastric emptying test (GET) and standardized nutrient challenge testing (NCT) were performed. Peripheral blood mononuclear cells isolated by density centrifugation and T-cells were quantified by flow cytometry. Duodenal 16S rRNA were targeted and abundance at the Operational Taxonomic Units (OTUs) level determined and relative abundance compared across groups utilising QIIMEII.

Results: 20/40 patients with chronic FGID symptoms reported NCWS compared to 2/20 controls. Overall FGID patients had an increased symptom response ($P < 0.001$) to the NCT compared to controls but no alterations of GET. FGID patients with NCWS had significantly increased gut homing cells compared to FGID patients without NCWS or controls (e.g. CD8+ 0.54, ±0.68 vs 0.17 ± 0.23 vs 0.13 ± 0.09, $P < 0.05$ FGID with NCWS vs controls). After adjusting for multiple testing, the relative abundance of 19 OTUs was different for FGID patients with vs without NCWS (Figure 1).

Summary & Conclusions: FGID patients (with and without NCWS) have an increased symptom response to NCT. The manifestation of NCWS in FGID is linked to an increase of gut homing immune cells and significant differences in the small intestinal MAM when compared to FGID without NCWS.

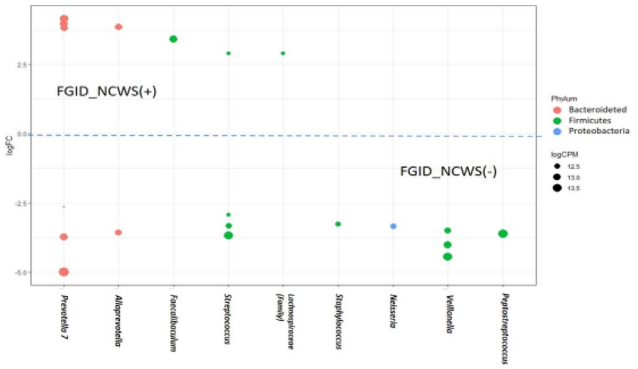


Figure 1: The significant differential abundance of OTUs (a false discovery rate threshold (FDR) < 0.001) between patients with unexplained (functional) gastrointestinal symptoms without wheat related symptoms (FGID_NCWS (+)) *vs.* FGID_NCWS (-). Upper axis represents OTUs with a log2 fold positive difference for FGID_NCWS (+) patients relative to FGID_NCWS (-) while the lower y-axis is the negative fold difference of the FGID_NCWS (+) relative to FGID_NCWS(-). Each point represents a single OTU coloured by phylum and grouped on the x-axis by taxonomic genus level, size of point reflects the log counts per million (logCPM) of abundances of taxonomic OTUs.

305 | The role of IL-1-dependent glial activation in the inflamed gut of mice and humans

Reiner Schneider; Patrick Leven; Stefanie Lück; Mariola Lysson; Bianca Schneiker; Johannes K. Hupa; Burkhard Stoffels; Joerg C. Kalff; Sven Wehner

Department of Surgery, University Hospital Bonn, Bonn, Germany

Background & Objectives: Enteric glial cells (EGC) modulate motility, maintain gut homeostasis and contribute to neuroinflammatory mechanisms in intestinal diseases including motility disorders. Interleukin (IL)-1 signaling is known to play a central role in intestinal inflammation activating various cell types including EGCs. We investigated the role of IL-1-triggered glia activation in intestinal neuroinflammation altering motility.

Methods: We studied the impact of IL-1 activated EGCs on disease progression in a mouse model of inflammation-induced motility disorder by applying various knock-out mouse lines deficient for key

components of the IL-1-signaling pathway including Myd88, IL-1R1, IL-1α and Il-1β. Histological analysis showed the impact on leukocytes infiltration and changes in the enteric nervous system. Further RNA-sequencing in these IL-1-deficient mouse lines and primary EGC cultures treated with IL-1b provided a detailed view on functional differences following IL-1 activation. Finally, human gut specimens were used to define the glial IL-1-activation in patients after enduring surgery and to test the therapeutic value of IL-1 blockage.

Results: Glial specific Myd88$^{-/-}$ and IL-1R1$^{-/-}$ mice are protected from inflammation-induced motility impairments and disease-typical leukocyte infiltration. RNA-Sequencing at early disease stages showed a distinct effect of glial IL-1-deficiency on gene clusters involved in neuronal activity and leukocyte migration. Subsequent RNA-Sequencing in primary EGCs treated with IL-1β showed the upregulation of many factors involved in migration and neuronal differentiation supported the in vivo findings. Further in vitro studies clarified the effect of EGCs on leukocyte migration and activation. Human jejunum specimens showed strong inductions of IL-1-signaling target genes after being mechanically activated during surgical procedures and ex vivo analysis using IL-1 signaling antagonists dampened inflammation and glia activation.

Conclusions: Glial IL-1 signaling is required for development motility impairments after intestinal inflammation in mice. IL-1 signaling blockage in EGCs is an effective treatment for inflammation-induced motility disorders and is a novel promising therapeutic approach.

311 | Exploring the interactions between the developing enteric nervous system and its surrounding tissues with an intestinal organoid model and single-cell sequencing

Elise Loffet[1]; Lisa Brossard[1]; Nicole Brown[2]; Nambirajan Sundaram[2]; Carine Bouffi[2]; Holly M. Poling[2]; Michel Neunlist[1]; Michael A. Helmrath[2]; Maxime M. Mahe[1,2]

[1]*Inserm UMR 1235 – TENS, University of Nantes, Inserm, Nantes, France;* [2]*Division of Pediatric General and Thoracic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*

Background: In the developing digestive tract, cellular interactions are essential to the development of digestive functions. One of the key regulators of the intestinal cellular microenvironment is the enteric nervous system (ENS). ENS progenitor cells (vagal neural crest cells or vNCCs) and gut endoderm co-develop during gut formation. However, the ENS role on intestinal development remain poorly understood. In this context, we hypothesized that the ENS contributes to the development of mesenchymal intestinal tissues.

Methods: To address our hypothesis, we used human intestinal organoids (HIO) produced from directed differentiation of human pluripotent stem cells (hPSCs), that were associated with vNCCs, to obtain innervated organoids (HIO + ENS). After generating HIO and HIO + ENS at different timepoints, we performed both "bulk" and single cell RNA sequencing to investigate how the presence of an ENS impacts development of mesodermal cell populations.

Results: Differential gene expression and ontology analysis demonstrated that HIO + ENS present increased expression of mesoderm and derivative tissues. In addition, we detected significant changes in ontologies related to muscle cell development and differentiation. These results were confirmed using a single cell sequencing approach in which we detected changes within mesoderm populations.

Conclusion: From our initial line of experimentation, these results strongly suggest that ENS progenitor cells have an impact on development and patterning of intestinal mesoderm-derived cells. These results need to be confirmed by further experimentation to decipher the cell fate transitions and the mechanisms involved in the gut mesoderm patterning.

316 | The relation between gastric motility, as assessed with the novel VIPUN balloon catheter, gastric emptying and nutrient load

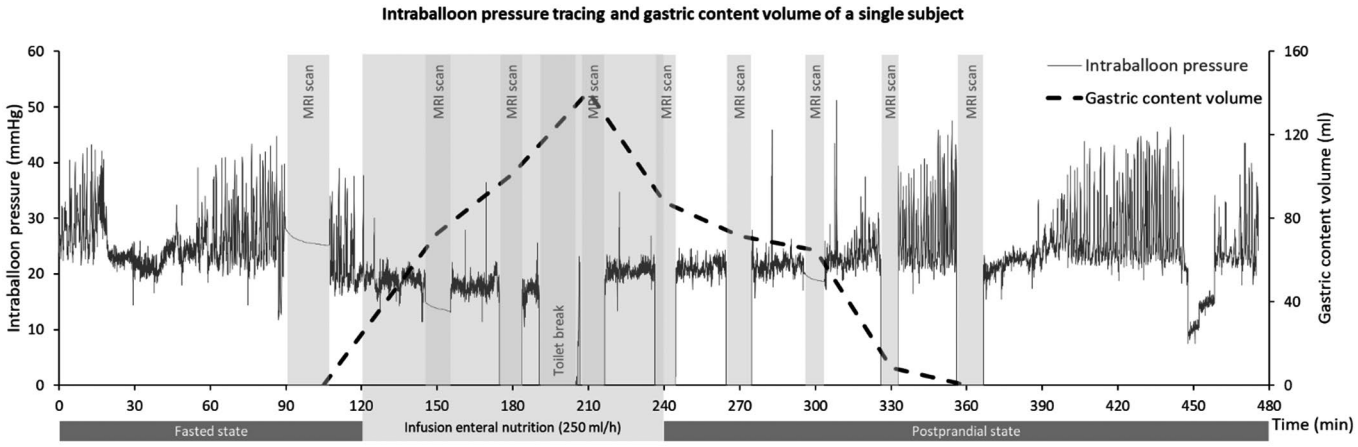
Nick Goelen[1]; Glynnis Doperé[1]; John Morales[2]; Sabine Van Huffel[2]; Vincent Vandecaveye[3]; Jan Tack[1]; Pieter Janssen[1,4]

[1]*TARGID, KU Leuven, Leuven, Belgium;* [2]*ESAT STADIUS, KU Leuven, Leuven, Belgium;* [3]*Department of Radiology, University Hospital Leuven, Leuven, Belgium;* [4]*VIPUN Medical, Boortmeerbeek, Belgium*

Background/Objectives: A neurohumoral feedback loop matches gastric outflow with the processing capacity of the small intestines through regulation of gastric motility. We recently developed the VIPUN Gastric Monitoring System that allows to continuously assess gastric motility using an inflated intragastric balloon mounted on a nasogastric feeding tube. We hypothesised that intragastric infusion rate of enteral nutrition (EN) dose-dependently inhibits gastric motility, exemplifying the neurohumoral feedback loop.

Methods: After an overnight fast, the VIPUN catheter was positioned with the balloon in the stomach. After inflating the balloon with 150 mL air, the catheter was connected to a pressure sensor. A Gastric Balloon Motility Index (GBMI) ranging 0 (no motility) to 1 (maximum contractile activity) was calculated from the pressure signal. Fasted motility was recorded for 2 hours, thereafter EN (1 kcal/mL) was infused for 2 hours, either at 25, 75 or 250 mL/h. Recording continued for an additional 4 hours. Gastric Content Volume (GCV) was quantified with magnetic resonance imaging every 30 minutes. Data are presented as mean(SD) with Bonferroni adjusted *P*-values.

Results: Nineteen healthy subjects were enrolled and 10 completed all three visits: 8 women, age: 33(14) years, BMI: 24.4(2.0) kg/m². Motility decreased during EN infusion at 250 mL/h (ΔGBMI = −0.42(0.12), $P < 0.0001$) and at 75 mL/h (ΔGBMI = −0.19(0.13), $P < 0.0001$), not at 25 mL/h (ΔGBMI = 0.03(0.22), $P = 0.99$). GCV at the end of EN infusion was greater at 250 mL/h (119.8(70.9) ml) vs 75 mL/h (9.8(16.6) ml, $P < 0.0001$). Motility was lower when EN was present in the stomach (GCV > 0 mL) relative to an empty stomach (0.27(0.24) vs 0.62(0.22), $P < 0.0001$).



Original intraballoon pressure was recorded for 8 hours and is plotted on the left y-axis. Recording was interrupted during MRI scan sessions as indicated. The gastric content volume, assessed with MRI is plotted on the right y-axis. Nine MRI scans were performed. The period in fasted state, during nutrient administration and the postprandial period are indicated.

Conclusions: Gastric motility was inhibited with increasing EN infusion rate and increasing GCV. Postprandial motility remained lower until emptying was completed. Phasic gastric motility is an important driver of gastric emptying which is inhibited through duodenal exposure to relatively large doses of nutrients.

320 | Human intestinal smooth muscle progenitors generate orientated muscle layers for intestinal regeneration

Silvia Perin[1]; Conor McCann[1]; Luca Peruzza[2]; Dipa Natarajan[1]; Paolo De Coppi[1]; Nikhil Thapar[1,3]
[1]*UCL GOS Institute of Child Health, London, UK;* [2]*Southampton University, Southampton, UK;* [3]*Queensland Children's Hospital, Brisbane, Australia*

Background/Objectives: Several severe gastrointestinal disorders involve functional and/or structural disruption of the neuromuscular compartment. Therefore, cell therapy could be beneficial for the treatment of such diseases. The aim of this project was to identify a pool of intestinal smooth muscle progenitor cells (SMPCs) and determine their ability to generate functional smooth muscle for regenerative medicine purposes.

Methods: SMPCs were isolated from foetal and paediatric intestine and characterized by immunostaining, flow cytometry, SNP arrays and RNA-seq analyses. In vitro, their ability to produce smooth muscle cells was examined via activation/inhibition of the TGFß pathway, by immunostaining, WB, qPCR, RNA-seq and calcium imaging. Finally, SMPCs were used to regenerate the muscular compartment of decellularised foetal human intestine in ex vivo culture, and analysed by immunohistochemistry.

Results: Cultured SMPCs demonstrated high proliferative capacity (66.4% ± 9.1 $Ki67^+$), maintenance of genomic stability, a mesenchymal origin (98.6% ± 1.3 $CD90^+$) and features in common with pericytes (88.6% ± 10.6 $NG2^+$, 98.0% ± 1.7 $CD146^+$ and 51.6% ± 39.8 $PDGFR\beta^+$). Immunostaining, RNA-seq, qPCR and WB data targeting the expression of SM22, Calponin and MYHC confirmed smooth muscle differentiation of SMPCs that varied depending on the levels of TGFß activation. Upon TGFß inhibition, cells displayed some level of differentiation due to an activation of the IGF-pathway as evidenced by RNA-seq analyses. Differentiated SMPCs also exhibited calcium transients in response to carbachol. SMPCs, when seeded onto decellularised scaffolds, distributed in two orthogonal layers of $SM22^+$/$Calponin^+$ cells, matching the anatomical structure of healthy intestinal muscle layers.

Conclusions: Our results confirm that SMPCs generate mature smooth muscle cells in vitro, mostly via activation of the TGFß pathway. Ex vivo, these cells have the capacity to self-organize in two orthogonally aligned smooth muscle layers. Future experiments are investigating the functionality of these layers via organ bath contractility, plus the involvement of the IGF-pathway. Thus, SMPCs show good potential for the treatment for enteric smooth muscle disorders.

324 | Estrogens induce visceral hypersensitivity and low-grade inflammation in male rats after early life stress

Alison Accarie; Lucas Wauters; Joran Toth; Ricard Farré; Jan Tack; Tim Vanuytsel
Ku Leuven Translational Research Centre for Gastrointestinal Disorders

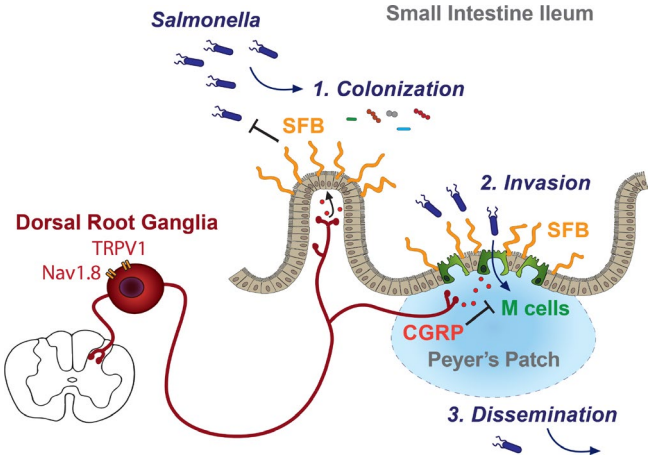
Objective: Functional gastro-intestinal disorders (FGID) are twice as prevalent in women than in men. Furthermore, stressful events are reported as one of the triggers for FGID symptoms. Recently, our group identified the Biobreeding rat (BB-rat) as a spontaneous model of FGID. In our previous study we demonstrated that only females displayed a gastrointestinal hypersensitive phenotype after early life stress, which was prevented by ovariectomy. We aimed to further characterize the interplay between estrogen and early life stress on the onset of colonic sensitivity, immune infiltration and permeability by exposing stressed males to estrogens.

Methods: New born male BB-rats (n = 7/group) were separated from their mother from d2 to d14, 3 h/d. Basal colonic sensitivity was assessed at d90 by measuring the visceromotor response (VMR) to isobaric (from 15 to 60 mm Hg) distensions. Estrogen or vehicle was subcutaneously injected every 4 days for 12 days. After the third injection, anxiety-like symptoms, assessed with the marble burying test and colonic sensitivity were measured. After the fourth injection, intestinal and colonic permeability, mast cell and eosinophil infiltration were measured with Ussing chamber studies, Chromotrope2R staining and Mast Cell Protease type 2 immunostaining respectively.
Results: Estrogen induced an increased visceromotor response to colorectal distension (Fig. 1) in males previously subjected to early life stress. Moreover, estrogen exacerbated anxiety-like behavior (3.6 ± 0.88 vs 10.40 ± 2.04 marbles buried; $P < 0.05$) and promotes the mucosal infiltration of eosinophils (527.7 ± 106.8 vs 1257 ± 181.1/mm^2; $P < 0.05$) and mast cells (356.3 ± 70.37 vs 842.6 ± 100.1/mm^2; $P < 0.05$) in the jejunum, while in the colon only the eosinophil density was significantly higher (58.91 ± 8.72 vs 101.4 ± 69.76 $P < 0.05$). Intestinal and colonic permeability were not altered by estrogen administration.
Conclusion: Our results point out that estrogen injection in adult males who underwent early life stress, reproduced the same intestinal features as in stressed females and identify estrogens as an important trigger for the onset of FGID pathogenesis in females with early life trauma.

335 | Gut-innervating nociceptor neurons regulate Peyer's patch Microfold cells and Segmented Filamentous Bacteria levels to protect against *Salmonella* infection

Nicole Lai[1]; Melissa Musser[1]; Felipe Pinho-Ribeiro[1]; Pankaj Baral[1]; Amanda Jacobson[1]; Donggi Paik[1]; Salima Soualhi[2]; Jun Huh[1]; Meenakshi Rao[2]; Isaac Chiu[1]
[1]*Harvard Medical School, Boston, MA, USA;* [2]*Boston Children's Hospital, Boston, MA, USA*

Background/Objectives: Pain-mediating sensory neurons, called nociceptors, innervate the gut and respond to noxious stimuli by initiating protective responses including inflammation and diarrhea. However, the role of gut-innervating nociceptors in crosstalk with the immune system is unclear. We hypothesized that nociceptor neurons provide a crucial alarm system to regulate host defense against the enteric pathogen *Salmonella typhimurium*.
Methods: Using genetic, pharmacological, and anatomical approaches, we depleted TRPV1 and Nav1.8 nociceptors in mice to examine their role in *Salmonella* infection. Using 16S rRNA sequencing, scanning electron microscopy, immune and epithelial analyses, we examined bacterial composition, Peyer's patch (PP) Microfold cells, and select immune populations in nociceptor-depleted mice.
Results: Dorsal root ganglia (DRG) nociceptors critically protect against *Salmonella* infection. Nociceptor depletion resulted in higher *Salmonella* colonization, invasion, and dissemination from the gut. Several myeloid and lymphoid immune cell populations were unchanged in the lamina propria and PP between nociceptor-depleted and control mice. In the follicle-associated epithelia of ileum PP, nociceptor neurons suppressed the density of microfold cells, which are critical entry points of *Salmonella* invasion. Downstream of microfold cells, nociceptors maintained levels of Segmentous Filamentous Bacteria (SFB), a gut microbe residing on ileum villi and PP epithelia that was necessary and sufficient for nociceptor-mediated protection against *Salmonella*. TRPV1+ nociceptors directly responded to *Salmonella* by releasing calcitonin gene-related peptide (CGRP), a neuropeptide that modulates microfold cells and SFB levels and is critical for protection during *Salmonella* infection.
Conclusions: Our data indicates that TRPV1+ DRG neurons regulate PP microfold cell levels through CGRP, which in turn mediates SFB attachment to PP epithelia to limit *Salmonella* invasion. These findings reveal a major role for nociceptor neurons in sensing and defending against enteric pathogens. Deciphering how host sensory neurons crosstalk with pathogenic bacteria has implications for development of therapies to control pain and to treat enteric infections.



339 | Role of colonic mucosal micro-RNA and host-microbiome interactions in irritable bowel syndrome (IBS)

Swapna Mahurkar-Joshi[1]; Elizabeth J. Videlock[1]; Jonathan P. Jacobs[1,2]; Abhishek Verma[1]; Venu Lagishetty[2]; Ariela Khandadash[1]; Dimitrios Iliopoulos[3]; Charalabos Pothoulakis[3]; Emeran A. Mayer[1]; Lin Chang[1]
[1]*Division of Digestive Diseases, Department of Medicine at UCLA, G. Oppenheimer Center for Neurobiology of Stress and Resilience;* [2]*Division of Digestive Diseases, Department of Medicine at UCLA, UCLA Microbiome Center;* [3]*Division of Digestive Diseases, Department of Medicine at UCLA, UCLA Center for Inflammatory Bowel Diseases*

Background/objectives: Alterations in miRNA and microbiome are putative factors in irritable bowel syndrome (IBS) pathogenesis.

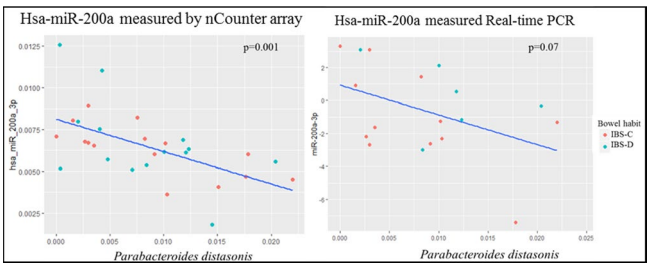
However, colonic mucosal miRNA pathways and host-microbiome interactions have not been well studied in IBS. Aims of this study were to: 1) identify differentially expressed miRNAs and their targets in IBS compared to HCs, 2) compare mucosal microbial composition between IBS and HCs, and 3) identify correlations between mucosal miRNA and microbiome.

Methods: Twenty-nine Rome III+ IBS patients (15 IBS with constipation [IBS-C, 66% women], 14 IBS with diarrhea [IBS-D, 43% women], and 15 age-matched HCs [50% women]) underwent sigmoidoscopy with biopsies. MiRNA expression was assessed using nCounter array and RT-PCR, mRNA by QuantSeq, and microbiome by 16S rRNA gene sequencing. MiRNAs were silenced or overexpressed in NCM460 normal colonic epithelial cell lines and pathway analysis was performed.

Results: (a) miRNA expression: Four miRNAs were differentially expressed in IBS compared to HCs (FDR < 10%). There was downregulation of hsa-miR-338-3p and hsa-miR-219a-5p in IBS (and IBS-C) vs HCs, which was validated. Inhibition of hsa-miR-338-3p in cells showed a downregulation of mitogen activated protein kinase (MAPK) inhibitors, potentially leading to activation of MAPK pathway, which plays a role in visceral hypersensitivity. Silencing hsa-miR-219a-5p in cells resulted in downregulation of proteasome-complex genes, including PSMB1, associated with barrier function and, mitochondrial metabolism genes, including ATP5F1A, associated with neurodegenerative diseases. (b) Mucosal microbiome: Microbial richness and diversity were lower in IBS vs HCs (P = 0.005 and 0.03), with an increased abundance of phylum Verrucomicrobia and genus Prevotella-2 in IBS vs HCs (FDR < 5%). (c) miRNA–microbiome correlations: hsa-miR-200a-3p was higher in IBS compared to HCs (P < 0.05) and negatively correlated with commensal bacteria *Parabacteroides distasonis* (FDR = 0.03). This was validated by RT-PCR (Figure 1). Overexpression of hsa-miR-200a-3p in cells upregulated immune system and cell adhesion pathway genes including GPRC5A, which are used in host defense against microbial infection, suggesting an increased miRNA-mediated suppression of commensals in IBS.

Conclusion: Mucosal miRNAs independently or in association with mucosa-adherent microbiome may play a pathophysiologic role in altered visceral sensitivity, barrier function and immune regulation via MAPK, neuronal and immune pathways.

Figure 1 Negative correlation between hsa-miR-200a-3p and *Parabacteroides distasonis* in IBS. Negative correlation between hsa-miR-200a-3p measured by A) Nanostring nCounter assay B) Real-time PCR and *Parabacteroides distasonis*; IBS-Constipation; IBS-D, IBS-Diarrhea; *P*, correlation *P* value.



341 | Predictors of treatment response to diet or medication in a large controlled trial in primary care IBS

Karen Van den Houte; Celine Tack; Jessica Biesiekierski; Linde Besard; Jolien Schol; Esther Colomier; Florencia Carbone; Jan Tack

TARGID

Objective: Irritable bowel syndrome (IBS) is one of the most common conditions encountered in clinical practice, not only by gastroenterologists but also by primary care physicians. Although IBS is highly prevalent, it represents a heterogeneous group of patients, which can be subdivided according to symptom characteristics and demographic factors. Belgian IBS patients are most frequently treated with the musculotropic spasmolytic agent otilonium bromide (OB). Reports from specialist care showed efficacy of a low FODMAP diet, but its use in primary care IBS is understudied. Our aim was to evaluate whether clinical predictors are associated with treatment responses in primary care IBS.

Methods: Sixty-three Belgian physicians recruited newly diagnosed IBS patients who were randomized in a pragmatic open parallel-group trial to OB 60 mg t.i.d., or a simple FODMAP lowering diet (IBS diet), provided via a smartphone application. Before and after 8 weeks of treatment, patients completed questionnaires evaluating demographics, stool types, Rome IV criteria, IBS-Symptom Severity (IBS-SSS), anxiety (GAD), depression (PHQ9) and somatization (PHQ15). Patients with a 50-point drop on IBS-SSS were considered a responder.

Results: 362 primary care patients (70% fulfilling Rome IV criteria, Rome+) were randomized to OB (n = 179; 77% females; mean age 41.6 ± 1 year) or IBS diet (n = 182; 75% females; mean age 41.0 ± 1 year). Respectively 59% and 70% of the patients treated with OB and diet were responders. In the OB group, responders had higher somatization scores compared to non-responders (10.5 ± 0.5 vs 9.0 ± 0.4, P = 0.01), but both groups had comparable demographic and other clinical characteristics. Responders to the diet were younger than non-responders (39 ± 1 year vs 44.6 ± 2 years, P = 0.03). Diet response was not determined by stool type, but Rome+ patients were more likely to respond to the diet compared to Rome- (77% vs 54%, P = 0.002).

Conclusion: In a large primary care IBS cohort, response to OB was associated with higher somatization scores and response to diet with a younger age and fulfilment of the Rome IV criteria.

346 | Role of muscularis macrophages in the maintenance of NOS1+ enteric neurons

Sihan Ji[1]; Magdalini Mischopoulou[1]; Alec Wright[1]; Jose Silva[1]; David Linden[1]; Tamas Ordog[1]; Lei Sha[2]; Gianrico Farrugia[1]; Gianluca Cipriani[1]

[1]*Mayo Clinic, Enteric NeuroScience Program;* [2]*Department of Neuroendocrine Pharmacology, School of Pharmacy, China Medical University*

Introduction: Macrophages in the muscularis propria of the gut (MMs) contribute to the maintenance of enteric neurons (ENs). The colony stimulating factor 1 receptor (CSF1R) represents a key regulator of MMs maintenance and constitutive loss of CSF1 results in few MMs and increased number of ENs. However, it remains unclear whether this is due to developmental or long-term loss of MMs.

Aim: Determine the effect of a transient depletion of MMs on ENs in adult mice.

Methods: 12-week old C57BL/6 mice were given daily i.p. injections of anti CSF1 neutralizing antibody (CSF1Ab) (100 or 200 μg) for 7 days. Another group of mice were maintained for 6 weeks following CSF1Ab to allow MM restoration. IgG-treated and untreated mice were used as controls. MMs were isolated from tissues as $CD45^{+}/CD11b^{+}/F480^{+}$ cells by fluorescence-activated cell sorting (FACS). Expression of selected genes in isolated MMs was compared to source tissues by RT-PCR. Gene expression changes following MM restoration were compared to untreated mice by RNAseq.

Results: CSF1-r was expressed exclusively in MMs and CSF1Ab treatment depleted almost 80% of total MMs (IgG treated; 131.33 ± 11.08 cells per field; CSF1Ab: 31 ± 2.94 cells per field). In mice treated with CSF1Ab the numbers of nitric oxide synthase 1^{+} ($NOS1^{+}$) (IgG treated; 23.36 ± 4.94 cells per field; CSF1Ab: 11.61 ± 4.40 cells per field) and total ENs were reduced (IgG treated: 99.25 ± 9.72 cells per field; CSF1Ab; 75.4 ± 5.6 cells per field) compared to IgG-treated mice. Notably, other cell types, such as ICC and $PDGFRA^{+}$ cells were not affected. After 6 weeks from the cessation of CSF1 treatment, the same number of MMs was found compared to IgG treated mice (135.34 ± 12.76 cells per field), with the same tissue-specific phenotype and the number of $NOS1^{+}$ ENs was restored (24.56 ± 6.56 cells per field).

Conclusions: MMs depletion, following CSF1Ab treatment, associated with loss of $NOS1^{+}$ ENs, suggesting a role for MMs in the maintenance of this subtype of ENs.

Supported by DK68055 and ANMS grant and AGA grant#36.

355 | Metabolomic and microbial profiles identify subsets of diarrhea predominant irritable bowel syndrome

Sean M.P. Bennet[1]; Giada De Palma[2]; Premysl Bercik[2]; Alan E. Lomax[1]; Stephen J. Vanner[1]; David E. Reed[1]

[1]*GIDRU, Queen's University, Kingston, Canada;* [2]*Farncombe Institute, McMaster University, Hamilton, Canada*

Background/objectives: Irritable bowel syndrome (IBS) patients are subtyped by predominant bowel habit rather than pathophysiological mechanisms and this may underlie challenges in identifying targets for effective treatments. We aim to identify IBS patient subgroups using metabolomics and microbial analysis.

Methods: Symptom history, stool and urine were collected from 30 diarrhea (IBS-D) and 30 constipation predominant (IBS-C) IBS patients (Rome IV). Liquid Chromatography-Mass Spectrometry quantified 130 metabolites in stool and urine. The GA-map™ Dysbiosis Test (≥300 bacteria) identified stool microbial composition. Multivariate discriminatory analysis assessed metabolomics and microbial profiles.

Results: Within IBS-D and IBS-C, combined stool/urine metabolomic profiles of patients with dysbiosis-like (DL) IBS (onset following antibiotics, enteric infection, or travel) were distinct from patients with non-DL IBS onset (IBS-D R2 = 0.7, Q2 = 0.5; IBS-C R2 = 0.5, Q2 = 0.4). Glutamic acid, α-ketoglutaric acid, C9 and phenylethylamine were main metabolites driving separation in IBS-D; pyruvic acid, putrescine and taurine were main metabolites driving separation in IBS-C. Multivariate analysis of bacterial profiles could only discriminate DL vs non-DL onset in IBS-D (R2 = 0.8, Q2 = 0.4); Phascolarctobacterium sp. and Lactobacillus spp. were the main bacterial probes driving the separation. Among patients with DL IBS, stool metabolomic profiles of 7 IBS-C were distinct from 8 IBS-D patients (R2 = 0.9, Q2 = 0.8) with metabolites lysine, asparagine, C4-OH and C10:1 driving the separation. No differences were found within non-DL IBS patients. There were no distinct metabolomic profiles between high and low levels of anxiety, depression or stress within IBS-D or IBS-C.

Conclusion: IBS-D patients with a history suggesting alteration in the microbiota prior to onset of the disorder had different metabolomic and bacterial profiles compared to IBS-D patients who did not have this history. The metabolomic and microbiota profiles could provide potential biomarkers for patients with this pathophysiological background. Moreover, the metabolome may contain metabolites produced by the microbiota that plays a pathophysiological role in this subgroup of patients and requires further study.

358 | Distinct 'low' and 'high' threshold mechanosensors exist in enteric glia and neurons, respectively—Deemed to be an important regulatory pathway in peristalsis

Elvio Mazzotta[1]; Egina Villalobos-Hernandez[1]; Iveta Grants[1]; Jonathon McClain[2]; Brian Gulbransen[2]; Alan Harzman[1]; John Grider[3]; Fievos L. Christofi[1]

[1]*The Ohio State University Wexner Medical Center;* [2]*Michigan State University;* [3]*Virginia Commonwealth University*

Background/Objective: Ca^{2+} signals in enteric glia (EGC) are required for normal motility and are implicated in GI Diseases and Disorders (Gastroenterology, 2018). Mechanical forces encountered during peristalsis such as touch, pressure, compression, flow or stretch trigger Ca^{2+} waves in glia in mice and humans, and responses are altered by inflammation. Touch activation of a single glia can facilitate local non-cholinergic contraction and glia modulates peristaltic waves. We sought to further investigate mechanosensation in glia and neurons.

Methods: A 1 second touch to trigger a Ca^{2+} wave was applied to a single glia or neuron in steps of 1-to-19 μm with a fire-polished glass micropipette tip (1.5, 4.5 μm) controlled by a Piezo-micromanipulator in human EGC (hEGCs) or Ca^{2+} reporter mice for glia [GCaMPtdTflox::Sox10Cre ERT2] and neurons [GCaMPtdTflox(−/−)::Wnt1CreERT2]. Atropine and nicardipine limited muscle contractions.

Results: Touch stimulation of a glial cell triggers a glial Ca^{2+} wave in Sox10 (N = 10, n > 400 trials) or Wnt1 mice (N = 4) not neurons (Fig. 1). Touch of a single glial cell triggers local contraction underneath the ganglion that depends on glial Ca^{2+} wave propagation. Touch/pressure stimulation causes a graded-response (sigmoid curve) in glia. The threshold stimulus is ≤1 μm-step in glia compared to 13.1 ± 0.7 μm-step for a sensory neuron ($P < 0.00001$, n = 10). An all-or-none response occurs in neurons with a threshold/max of 13.1 ± 0.7 μm-step (n = 10). Strength of response in a neuron is 4-fold higher than glia ($P < 0.0001$). The N-type Ca^{2+} channel inhibitor Ω-conotoxin-GVII (0.2 μM) blocked touch responses in 4/10 neurons and 0/11 glia. The Piezo-channel inhibitor GsMtx4 (5 μM) did not inhibit glial Ca^{2+} waves (propagation or strength) in mice ($P > 0.10$, N = 4) or hEGCs (N = 3, $P = 0.87$), even though >90% hEGCs responded to Yoda1 (20 μM, N = 3), express Piezo 1,2 mRNA and proteins (N = 4). Inhibition of Calcium Sensor Receptors (NPS153, 20 μM) reduced Ca^{2+} wave propagation 2.8 fold (n = 11 trials, $P < 0.0001$).

Conclusions: Data provides new insights into mechanosensation – Distinct 'low' and 'high' threshold mechanosensors exist in glia and neurons, respectively, that may represent an important regulatory mechanism of peristalsis (R01-DK113943).

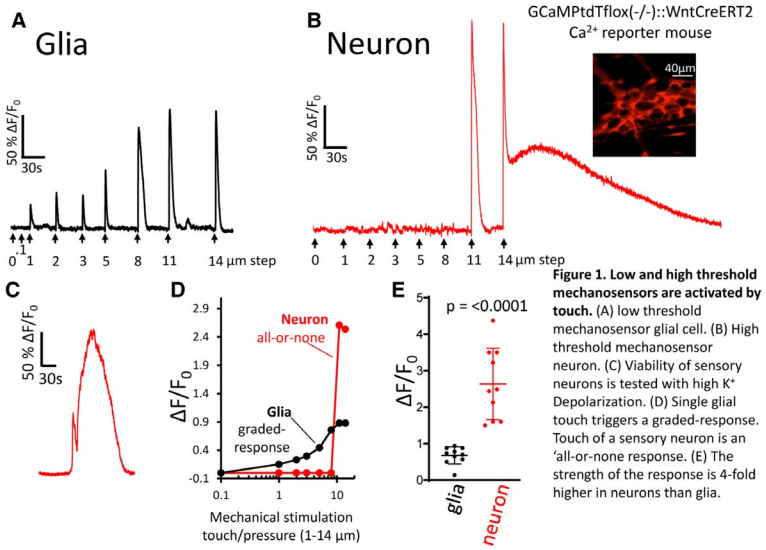


Figure 1. Low and high threshold mechanosensors are activated by touch. (A) low threshold mechanosensor glial cell. (B) High threshold mechanosensor neuron. (C) Viability of sensory neurons is tested with high K^+ Depolarization. (D) Single glial touch triggers a graded-response. Touch of a sensory neuron is an 'all-or-none response. (E) The strength of the response is 4-fold higher in neurons than glia.

360 | Glial endothelin-type B receptor is a potential pathogenic mechanism in postoperative ileus

Egina Villalobos-Hernandez; Elvio Mazzotta; Iveta Grants; Nick Paeglis; Kyle Hopkins; Reiner Schneider; Sven Wehner; John Grider; Brian Gulbransen; Fievos L. Christofi

The Ohio State University Wexner Medical Center, Columbus, Ohio, United States; University of Bonn, Bonn, Germany; Michigan State University, East Lansing, Michigan, United States

Background/Objectives: Our earlier study identified endothelin-type B receptor (ETB-R) in enteric glia that may contribute to inhibitory modulation of motility. Other studies suggested a pathogenic role in inflammatory conditions. We investigated the expression, distribution and function of glial ET-1/ETB-R signaling in glia and tested the hypothesis that ETB-R signaling is a pathogenic mechanism in postoperative ileus (POI) associated with inflammation.

Methods: We studied ETB-R expression in Tg(Ednrb(ETB-R)EGFP) EP59Gsat/Mmucd reporter mice by co-labeling for EGFP$^+$ cells, neurons (HuC/D$^+$), glia (s100β), ICC (c-Kit), smooth muscle (αSMA), enteroendocrine cells (EE,CgA); Ca^{2+} waves in GCaMPtdTflox::Sox10Cre ERT2 mice and GCaMPtdTflox(-/-)::Wnt1CreERT2 Ca^{2+} reporter mice; and human enteric glial cultures (hEGCs). In a mouse POI model, the ETB-R antagonist BQ788 (i.p. 1 mg/kg) was administered 7 consecutive days prior to gut surgical manipulation to evaluate impact on inflammation 24 hours later.

Results: Throughout the GI tract (esophagus to large intestine) ETB-R was expressed exclusively in glia in myenteric ganglia, and was absent from neurons, ICC's, smooth muscle or EE cells (Fig. 1). Glial mRNA for ET-1 and ETB-R were identified in RiboTag mice (N = 6). ET-1-immunoreactivity was strongly expressed in varicose nerve fibers densely innervating myenteric ganglia in intimate contact with glia expressing ETB-R. The ETB-R agonist Sarafotoxin-S6c induced Ca^{2+} waves in a majority of glia in hEGCs (N = 3), Sox10 (N = 8) and Wnt1 mice (N = 3, >80% tdT$^+$ glia/ganglion) not neurons (<2%). Responses in glia are insensitive to 1 μM TTX (n = 11 ganglia) but were blocked by BQ788 (1-3 μM, n = 10 ganglia, $P < 0.01$). In POI, reactive glia show ETB-R up regulation ($P < 0.001$), hypersensitivity to SaTX (at 1 nM, 3 nM; $P < 0.001$) and enhancement in ET-1 dependent neuron-to-glial communication activated by high K$^+$ (N = 4, $P < 0.001$). In vivo administration of BQ788 reduced by 2.2 fold leukocyte infiltration (MPO$^+$ cells; $P < 0.0001$, N = 4 mice each/sham vs POI ± BQ788).

Conclusions: ET-1/ETB-R signaling is a unique feature of enteric glia throughout the GI tract and is a potential pathogenic mechanism of POI. An ETB-R antagonist may be protective in POI (R01-DK113943 & Dean's Grant).

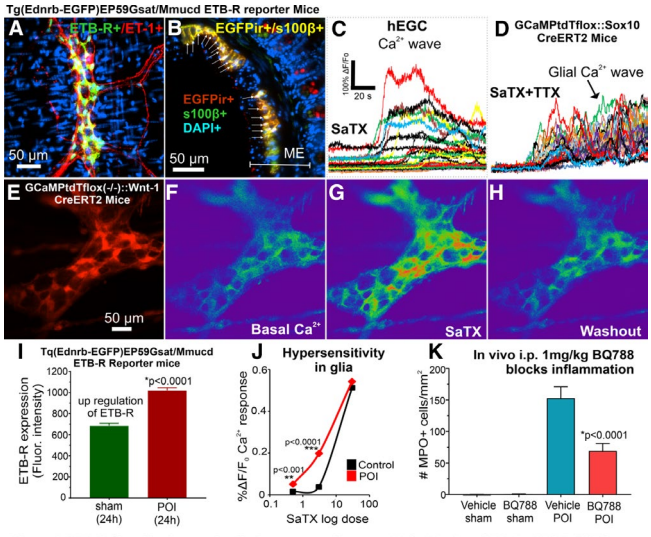


Figure 1. ETB-R Signaling in enteric glia in mouse and human. (A) Co-labeling of ET-1 in ETB-R EGFP reporter mice to show ET-1 nerve fibers in close proximity to ETB-R positive glia. (B) EGFP-immunoreactivity is restricted to glia in the ETB-R reporter mice; paraffin cross-section of small bowel. (C) The ETB-R agonist SaTX triggers a Ca^{2+} wave in hEGCs. (D) SaTX triggers a Ca^{2+} wave in the presence of TTX in mouse EGCs of the myenteric plexus in Sox10 Ca^{2+} reporter mice. (E) Image of tdT+ cells in the GCaMPtdTflox(-/-)::Wnt1CreERT2 mice used to study glial ETB-R receptors. (F-H) Pseudocolor images to show response to SaTX in glia. (I) ETB-R expression is up regulated in POI (24 h after gut manipulation). (J) Hypersensitivity of the ETB-R receptor in POI. (K) In vivo administration of the ETB-R antagonist BQ788 significantly reduces gut inflammation in the muscularis externa by decreasing leukocyte infiltration, measured by MPO (myeloperoxidase)+ cells / mm^2.

370 | Increased symptom and brain responses to intragastric administration of FODMAPs in patients with irritable bowel syndrome vs healthy subjects

Imke Masuy[1]; Jie Wu[1,2]; Heather Fitzke[3]; Jessica Biesiekierski[4]; Jan Tack[1]; Lukas Van Oudenhove[1]

[1]*Translational Research Center for Gastrointestinal Disorders, KU Leuven, Belgium;* [2]*Central South University, Changsha, China;* [3]*Wingate Institute for Neurogastroenterology, Queen Mary University of London, UK;* [4]*LaTrobe University, Melbourne, Australia*

Background/objective: Visceral hypersensitivity to colonic distention after acute FODMAP intake, rather than excessive gastrointestinal gas and water production, trigger symptoms in IBS. The current study investigated the brain responses to acute intragastric administration of FODMAPs.

Methods: This randomized, double-blind, cross-over study was conducted in healthy controls (HC) and IBS patients and consisted of 3 study visits during which a test solution was intragastrically infused during MR brain scanning. The solutions tested were fructans (40 g/500 mL saline), glucose (40 g/500 mL saline) and saline (500 mL). Abdominal symptoms, vigor, fatigue, positive and negative affect scores were collected at regular time points. Symptom data were analysed using linear mixed models, and brain data were analysed using pharmacological MRI models within a mask of pain-responsive regions derived from the NeuroSynth meta-analysis repository.

Results: Thirteen HC (age: 31 ± 12 years; BMI: 22.3 ± 1.75) and thirteen IBS patients (age: 27 ± 7 years; BMI: 24.0 ± 4.20) completed the study. Patients reported more bloating, cramps and flatulence compared to HC over all solutions ($P < 0.005$). For bloating, no differences between fructans, glucose and saline were observed in both groups ($P > 0.15$). Fructans induced more flatulence than glucose ($P = 0.013$) and more cramps compared to saline ($P < 0.001$) in both groups. Pain was higher following fructans compared to glucose in IBS ($P = 0.002$). No significant differences were observed for vigor, fatigue, positive and negative affect between conditions or groups ($P > 0.05$). The increased pain perception after fructans in IBS was accompanied by stronger brain responses in key regions of the pain neuromatrix, including right anterior midcingulate and pregenual anterior cingulate cortex, left mid-insula, left angular and supramarginal gyrus, right putamen, right thalamus, right primary sensorimotor cortex and the periaqueductal gray matter ($p_{FWE\text{-}corrected} < 0.05$).

Conclusions: Enhanced pain responses to fructans compared to glucose in IBS patients are accompanied by increased brain responses in the pain neuromatrix.

379 | Serial diagnostic study: Effect of position and provocative tests on the diagnosis of oesophageal motility disorders by high-resolution manometry

Mark Fox[1,2]; Michael Hollenstein[1]; Simon Bütikofer[1]; Daphne Ang[3]; Henriette Heinrich[1]; Benjamin Misselwitz[1,4]

[1]*University Hospital Zürich and University of Zurich;* [2]*Digestive Function: Basel, Klinik Arlesheim;* [3]*Changi General Hospital;* [4]*Inselspital Bern and Bern University*

Background and Objectives: Standard high-resolution manometry (HRM) protocols are based on 10 single water swallows (SWS) acquired in the supine position; however, oesophageal motility can be assessed in the upright position and with provocative tests. This paper assesses the impact of position, rapid drink challenge (RDC) and solid test meal (STM) on the diagnosis of oesophageal motility disorders.

Methods: 72 healthy volunteers (20-76 years) and 366 consecutive patients (18-90 years) completed HRM with ten SWS in the supine and upright positions. RDC was performed twice, before and after STM. Diagnosis based on SWS in the supine position (Chicago Classification v3.0) was compared with results in the upright position and with provocative tests.

Results: Overall, diagnostic agreement in the supine and upright positions was present in 296/438 (67.6%) subjects (Table 1). This increased to 90.0% when ineffective oesophageal motility (IEM) was considered with normal motility. Integrated relaxation pressure was 4 mm Hg higher in the supine position. Reduced peristaltic vigour in the upright position resulted in a higher fraction of failed and ineffective swallows compared to the supine position ($P < 0.0001$). Applying a fixed 50% effective swallow threshold, 33.5% (93/278) of individuals with a "normal" HRM in the supine position were classified with "ineffective oesophageal motility" in the upright position. However, if appropriate thresholds were applied in both positions (50% and 20% in supine and upright positions), then diagnostic discordance was 12.5%. Concordance between SWS and STM for the diagnosis of IEM was similar in the supine than the upright position (Table 2). Conversely, there was a higher prevalence of false positive diagnoses of outlet obstruction in the supine compared to the upright position (16/20 vs 3/4 patients, $P = 0.0007$). The difference in concordance between SWS with STM was lower in the supine than the upright position (12/30 (40%) vs 12/15 (80%), $P = 0.014$).

Conclusion: Diagnostic agreement for oesophageal motility disorders based on SWS in the upright and supine positions was moderate, with frequent discordant findings for IEM and outlet obstruction. Clinical HRM studies can be performed in either position, using appropriate reference values. RDC or STM can resolve diagnostic discrepancies.

www.clinicaltrials.gov NCT02407938, NCT02397616.