Conference

The Enteric Nervous system in Health and Disease

Program and Abstract Book

Friday, May 29, 2026

Busto Arsizio, Sala Conferenze Museo del Tessile

Program

8.30 am – 9 am	**REGISTRATION AND POSTER SET UP**
9 am – 9.30 am	**WELCOME AND OPENING**

MORNING SESSION

PLENARY LECTURES
Chair: Cristina Giaroni

9.30 am - 10.30 am	**Prof. Pieter Vanden Berghe (Leuven Brain Institute):** *Complexity of the enteric nervous system as revealed by microscopy*
10.30 am - 11.30 am	**Prof. Werend Boesmans (Maastricht University):** *Enteric glia as an important mediator of enteric nervous system homeostasis in health and disease*
11.30 am – 12 am	***Coffee Break***

SHORT TALKS
Chair: Isabella Barbiero

12.00 pm - 12.20 pm	**Fabiana Miraglia (Scuola Normale Superiore, Pisa)** *α-Synuclein Aggregation Drives Significant Glia-neuronal Network Remodeling in Primary Enteric Cultures*
12.20 pm - 12.40 pm	**Giorgia Valetti (University of Insubria)** *Beyond the brain: uncovering gut barrier defect in CDKL5 deficiency disorder*
12.40 am – 2 pm	***Lunch Buffet And Poster Session***

AFTERNOON SESSION

SHORT TALKS
Chair: Erica Zamberletti

2.00 pm - 2.20 pm **Annalisa Bosi (University of Insubria)**
Gender-specific influence of antibiotic-induced dysbiosis and Lacticaseibacillus rhamnosus GG (ATCC 53103) effect on gastrointestinal motility in adolescent mice

2.20 pm - 2.40 pm **Francesca Terrin (University of Padova)**
beta-sitosterol beta-D-glucoside (BSSG) triggers intestinal inflammation in zebrafish and mouse models before the onset of neurodegeneration

PLENARY LECTURES
Chair: Werend Boesmans

2.40 pm - 3.40 pm **Prof. Cristina Giaroni (University of Insubria):**
The enteric nervous system at the crossroad of the microbiota-gut-brain axis

3.40 pm - 4.40 pm **Prof. Cecilia Giron (University of Padova):**
Communication between gut microbiota and neurons: the toll like story

4.40 pm – 5 pm **CLOSING REMARKS**

5:30 pm **POSTERS REMOVED**

Alphabetical List of Abstracts

Gender-specific influence of antibiotic-induced dysbiosis and *Lacticaseibacillus rhamnosus GG* (ATCC 53103) effect on gastrointestinal motility in adolescent mice

Annalisa Bosi[1], Alessandra Ponti[1], Elisabetta Moro[2], Sofia Faggin[3], Francesca Crema[2], Maria Cecilia Giron[3], Ilia Bresesti[4], Andreina Baj[1], Cristina Giaroni[1]

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Background. Early-life gut dysbiosis can induce enteric nervous system (ENS) vulnerability, contributing to disorders like Irritable Bowel Syndrome (IBS), which shows a higher clinical incidence in females. This study evaluated the long-lasting consequences of antibiotic-induced dysbiosis on gastrointestinal motility and the potentially restorative role of *Lacticaseibacillus rhamnosus* GG (LGG) in both male and female mice.
Methods. Dysbiosis was induced in C57BL/6 mice by administration of a cocktail of broad-spectrum antibiotics (0.5 mg/ml vancomycin, 1 mg/ml neomycin, 0.25 mg/ml ciprofloxacin and 1 mg/ml ampicillin, 0.015 mg/ml natamycin) from postnatal day (PND) 21 until PND 36, in drinking water. LGG ($2x10^9$ CFU) was administered daily from PND21 to PND42. Small intestine neuromuscular function was evaluated at PND43 and PND77.
Results. Antibiotic-induced dysbiosis significantly reduced intestinal transit efficiency in females at both PND43 and PND77, while males remained unaffected. Carbachol-induced cholinergic contractions were diminished in females at all ages, with LGG treatment providing restoration only at the PND77. In males, cholinergic impairment was transient (PND43) and was completely restored to control values by LGG administration. Electrical field stimulation (EFS)-induced contractions were reduced in both sexes at PND43, but LGG fully restored these levels only in male mice. This parameter remained downregulated only in female at PND77 and was restored to control values with LGG. At PND43, tachykinergic transmission, preprotachykinin-1 expression and substance P varicosity density were reduced exclusively in dysbiotic females. Inhibitory nitrergic relaxation was consistently impaired in females; conversely, males only exhibited a significant reduction later in adulthood at PND77.
Conclusions Early-life dysbiosis induces significant neuromuscular alterations that persist into adulthood, with females demonstrating a markedly higher and more enduring vulnerability. These findings correlate with the gender-specific prevalence of IBS. The restorative effects of LGG are highly sex-dependent, highlighting the necessity for personalized, gender-sensitive therapeutic strategies to effectively mitigate the permanent consequences of early microbial imbalances on the microbiota-gut-brain axis.

References
1. Bistoletti M et al. 2019 PLoS One 14:e0212856
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Selective Allosteric Modulation of GlyT1 by Obeticholic Acid: From Transporter Dynamics to Pain Models

Chiara D'Agostino[1], Tiziana Romanazzi[1], Daniele Zanella[2], Jennifer DeBerry[3], Giulia Casoli[1], Angela Di Iacovo[1], Cristina Roseti[1], Aurelio Galli[2], and Elena Bossi[1]

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Keywords: membrane transporter, neurotransmitter, SLC6, Glycine transporters, pain, bile acids

Bile acids (BAs) are increasingly recognized as signaling molecules within the gut–brain axis, regulating metabolic, immune, and neurochemical processes. Obeticholic acid (OCA), a semi-synthetic and potent farnesoid X receptor agonist, exerts systemic effects beyond the liver and intestine, including actions in the central nervous system. BAs can cross the blood–brain barrier and are also synthesized in the brain and contribute to synaptic modulation in multiple brain regions. Recently, we reported direct effects of OCA on the dopamine transporter (DAT) and other members of the SLC6 transporter family. OCA did not alter dopamine affinity or transport currents in DAT. Docking simulations identified two potential binding sites, one hypothetically stabilizing the inward-facing open conformation, suggesting a role for BAs as modulators of transporter function. Here, we examined OCA interaction with GlyT1 and GlyT2: the human and mouse transporters were expressed in Xenopus laevis oocytes, and activity was measured using two-electrode voltage clamp. OCA selectively modulated GlyT1 by altering substrate affinity without modifying maximal transport current, indicating an allosteric mechanism, whereas GlyT2 remained unaffected. The effect was conserved across species. Given the glial and neuronal localization of GlyT1 and GlyT2 and the role of the glycinergic system in nociception transmission, we investigate the possible role of OCA in this function. OCA treatment did not significantly affect nociceptive responses in vivo in either spared nerve injury (neuropathic pain) or Complete Freund's adjuvant (inflammatory pain) mouse models. These findings identify OCA as a selective allosteric modulator of GlyT1, extending the emerging role of BAs in neurotransmitter transporter regulation and underscoring the functional divergence between GlyT1 and GlyT2. Although OCA did not produce pain-relieving effects in neuropathic or inflammatory pain models, the results suggest that modulation of GlyT1 by OCA is insufficient to alter nociceptive responses under these experimental conditions but did not exclude a broader physiological role for GlyT1 regulation. Moreover, data indicates a potential therapeutic relevance for OCA in settings where slight and selective tuning of GlyT1 function may be strategic.

α-Synuclein Aggregation Drives Significant Glia-neuronal Network Remodeling in Primary Enteric Cultures

Fabiana Miraglia[1], Jessica Grigoletto[1], Caterina Sorteni[1], Eleonora Crocco[1], Alexia Tiberi[1], Rebecca Senter[2], Federico Cremisi[1], Antonino Cattaneo[1,3], A. Stewart Campbell[2], Emanuela Colla[4,1*]

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Keywords: Parkinson's Disease, alpha-synuclein, primary enteric cultures, neuron-glia network, antisense oligonucleotides

According to the body-first hypothesis, approximately 30% of Parkinson's disease (PD) cases originate outside the CNS, with early accumulation of alpha-synuclein (αS) inclusions in enteric neurons that precedes and spreads to the brain, ultimately leading to classical motor symptoms. In this study, we established primary enteric cultures (PECs) derived from the myenteric plexus of adult PrP A53T αS transgenic mice, a model of prodromal PD, to investigate the mechanisms underlying αS aggregation in the ENS and its impact on the glia–neuronal network. PECs develop a 3D, multilayered cytoarchitecture that recapitulates key features of the intestine, with smooth muscle cells forming a basal layer beneath a complex and interconnected glia–neuronal network. Although smooth muscle cells do not organize into distinct circular or longitudinal fibers, neurons and glial cells remain closely associated, forming ganglion-like clusters characterized by densely packed cell bodies and extensive neuritic processes. Functional analysis revealed that neurons in PECs exhibit evoked Ca^{2+} activity, responding robustly to depolarization, with no significant differences between genotypes. Exposure to exogenous pre-formed αS fibrils or insoluble spinal cord lysates from diseased A53T mice induced the accumulation of insoluble, neuronal αS inclusions, leading to pronounced remodeling of the glia–neuronal network. Specifically, αS aggregation was associated with a significant loss of HU^{+}-neurons and marked glial redistribution away from regions enriched in αS inclusions. Treatment with antisense oligonucleotides targeting αS effectively reduced endogenous αS expression and significantly decreased inclusion formation. However, this intervention did not fully restore the structural organization of the glia–neuronal network. In conclusion, PECs represent a versatile and physiologically relevant 3D model for dissecting early pathogenic mechanisms of PD within the ENS. Their cellular complexity and experimental accessibility make them a powerful platform for studying neuron–glia interactions and for evaluating therapeutic strategies targeting early enteric dysfunction in PD.

Endogenous hyaluronic acid regulates the mouse colon neuromuscular functions after induction of experimental colitis

Alessandra Ponti[1], Annalisa Bosi[1], Elisabetta Moro[2], Sofia Faggin[3], Maria Cecilia Giron[3], Francesca Crema[2], Cristina Giaroni[1]

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Background
Hyaluronic acid (HA) participates in both acute and chronic phases of inflammatory bowel disease (IBD) by influencing several cellular components of the gut microenvironment.[1,2] IBD is associated with increased HA deposition in the *muscularis propria* and myenteric plexus, where HA constitutes a perineuronal sheath, similar to perineuronal nets of the central nervous system.[2] This study aimed to evaluate endogenous HA as a modulator of the colonic neuromuscular function in mice undergoing dextran sodium sulfate (DSS)-induced colitis.

Methods
Male C57BL/6J mice received 2% DSS for 5 days. The HA synthesis inhibitor **4-MU (450 mg/kg)** was administered daily for 7 days. Colitis severity was assessed via DAI and imaging. The neuromuscular function was evaluated by measuring the efficiency of the gastrointestinal transit, and by recording proximal and distal colon motility through carbachol (CCh), Electrical Field Stimulation (EFS), and EFS-induced NANC relaxations.

Results
DSS caused systemic inflammation (weight loss, shortened colon, high DAI) and delayed GI transit, all of which were fully reversed by 4-MU. In the proximal colon, DSS significantly reduced carbachol and EFS-induced contractions; 4-MU successfully restored these responses and partially recovered tachykinergic contractions. Conversely, the injured distal colon showed preserved contractility but a significant reduction in nitrergic relaxation, which 4-MU failed to restore

Conclusions
These findings demonstrate that endogenous HA acts as a critical modulator of colonic neuromuscular function during experimental colitis**.** The study reveals a marked regional dimorphism in motor dysfunction: while HA synthesis inhibition with 4-MU effectively restores cholinergic and tachykinergic contractility in the non-inflamed proximal colon, it fails to rescue the impaired nitrergic relaxation in the injured distal colon. Overall, the ability of 4-MU to normalize systemic inflammatory markers and proximal dysmotility suggests that targeting HA deposition represents a promising therapeutic strategy for managing gastrointestinal motor disorders associated with inflammatory bowel disease.

References
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Altered gut homeostasis in *GBA1*-linked Parkinson's disease: evidence from a transgenic mouse model

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Keywords: GBA1, gut-brain axis, Parkinson's disease gut inflammation, microbiota dysbiosis

GBA1 gene encodes the lysosomal enzyme glucocerebrosidase (GCase), which catalyzes the hydrolysis of glucosylceramide (GluCer) to glucose and ceramide and the transglucosylation of cholesterol. Bi-allelic mutations of *GBA1* caused Gaucher disease (GD), a lysosomal storage disorder characterized by the lysosomal accumulation of GCase substrates within macrophages of the spleen, liver, bone marrow and of other tissues. GD can also present neurological manifestations and have a very broad spectrum of phenotypes. Heterozygous mutations in *GBA1* represent the most common genetic risk factor for Parkinson's disease (PD), a neurodegenerative disorder marked by dopaminergic neuronal loss and the presence of Lewy bodies, intraneuronal inclusions predominantly composed of aggregated *α*-synuclein (*α*-syn). GCase deficiencies promote *α*-syn accumulation and aggregation, exacerbating lysosomal dysfunction and neurodegeneration.
Emerging evidence highlights a bidirectional gut–brain axis, where gut physiology, microbiota composition, and enteric nervous system activity influence central nervous system function. Notably, inoculation of α-syn preformed fibrils in the duodenum of wild-type mice induces inflammation and reduces GCase activity, supporting a role for peripheral triggers in disease progression[1].
The present study investigates gut-brain interaction *GBA1* mutant conditions using *GBA*$^{-/-}$ *h*N370S transgenic mice[2], which express the human GBA1 N370S gene in a null murine Gba1 background6. Hematoxilin-eosin staining and transmission electron microscopy (TEM) revealed alteration in the length of intestinal villi and microvilli and in the width of tight and adherens junctions, suggesting impaired barrier integrity. These changes were associated with increased pro-inflammatory cytokines (MCAF, IL-1α, IL-1β, TNFα) and reduced short-chain fatty acids (SCFAs). Moreover, 16S rRNA analysis showed an increase in pro-inflammatory bacterial families, such as Muribaculaceae and Desulfovibrionaceae, and a decrease in SCFA-producing Firmicutes, including Lachnospiraceae.
Overall, these findings indicate the presence of an inflammatory response in the gut which could contribute to the disease etiopathogenesis in *GBA1*-linked PD.

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Title: β-sitosterol β-D-glucoside (BSSG) triggers intestinal inflammation in zebrafish and mouse models before the onset of neurodegeneration

Authors
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Abstract

Glucosylated-sterols can be synthetized endogenously, absorbed through the diet or derive from bacterial infection[1-3]. Their clinical relevance is currently underestimated, even though their imbalance has been associated with the risk of developing neurodegeneration throughout life[3,4]. We studied the detrimental effects elicited by dietary consumption of plant-derived β-sitosterol β-D-glucoside (BSSG), known to be associated with the occurrence of a complex disorder called Amyotrophic Lateral Sclerosis-Parkinsonism Dementia Complex (ALS-PDC)[5,6], to clarify its mode of action.

To achieve this goal, zebrafish larvae and adults, as well as adult mice, were treated with BSSG dissolved directly in the water or through customized food pellet, respectively. Since the intestine was identified as the primary target tissue, morphological and functional characterization, transcriptional analysis and sequencing of gut microbiota were carried out. Notably, *ex vivo* analysis of gut contractility was applied for the first time on zebrafish to assess intestinal neuromuscular response.

These broad analyses showed that BSSG caused intestinal inflammation in both zebrafish and mouse models. This previously unknown effect was evidenced by the presence of inflammatory response and altered gut dysmotility. Transcriptomic analyses revealed increased expression of inflammation-related genes in the intestine of both zebrafish and mice, while preliminary gut microbiota analyses suggested the onset of dysbiosis. Of note, transgenic and mutant zebrafish lines depleted of genes involved in glucocorticoids synthesis and activity[7,8] evidenced that BSSG likely interacts with the glucocorticoid receptor, potentially affecting its canonical anti-inflammatory activity.

We discovered a new set of pathways altered by dietary uptake of BSSG: this molecule initially leads to gut inflammation, altering intestinal morphology and functionality, and possibly determines the occurrence of neurodegeneration through interference with the well-known gut-brain axis.

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Title: “Mapping THES Gut Pathogenesis: Insights from a Novel ttc37 Knockout Zebrafish Model”

Key words:

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Background. Trichohepatoenteric Syndrome (THES) is an ultra-rare, life-threatening multisystem disorder caused by biallelic mutations in *TTC37* or *SKIV2L*, encoding core components of the SKI complex, a cofactor of the RNA exosome. The disease is characterized by severe intractable diarrhea requiring long-term parenteral nutrition, with high mortality primarily due to intestinal failure and recurrent infections. Despite its clinical implications, no curative treatment exists, and management is limited to supportive care.

Objective. The mechanisms through which TTC37 mutations give rise to such profound gastrointestinal dysfunction and high mortality have remained entirely unclear. The existing body of literature consists largely of clinical descriptions and case reports and provides no mechanistic explanation linking TTC37 deficiency to THES enteropathy. In particular, the contribution of the enteric nervous system in the determination of altered gut motility has not been investigated. This lack of mechanistic insight has significantly limited the identification of actionable therapeutic targets.

Methods and Results. To address this gap, we generated a *ttc37* knockout zebrafish line, representing the first *in vivo* model of THES. This model recapitulates key features of the human phenotype, including impaired intestinal motility consistent with defective peristalsis. Importantly, this platform enables *in vivo* investigation of disease mechanisms and provides a scalable system for screening candidate anti-diarrheal compounds targeting motility and secretion.

Conclusion. In conclusion, we established the first THES *in vivo* model, that could represents a novel and versatile platform for mechanistic studies and for the identification of therapeutic strategies aimed at alleviating THES symptoms.

Abstract title: "Beyond the brain: uncovering gut barrier defect in CDKL5 deficiency disorder"

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CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental disorder characterized by early-onset drug-resistant epileptic seizures, intellectual disability, motor and gastrointestinal (GI) impairment and caused by de novo mutations in the X-linked cyclin-dependent kinase-like 5 (*CDKL5*) gene. Given the high expression of CDKL5 in the brain and the main cognitive deficits of CDD patients, most of the research is focused on the protein role in the central nervous system. Despite 86% of patients reporting GI symptoms, eventually leading to gastrostomy with a great impact on the quality of life, the role of CDKL5 in the gut remains unexplored. To fill this gap, in this study we started to characterize the role of CDKL5 in the context of the intestinal epithelial barrier (IEB) as well as GI tract alteration caused by its absence in juvenile and adult pathogenic cohorts, including heterozygous females (HET) and knockout males (KO). After demonstrating the presence of Cdkl5 in the IEB, we next defined the phenotypic GI landscape of juvenile CDD mice. We observed that both KO males and HET females present reduced body weight and longer GI tract compared to the respective WT cohort. We also extended the analysis to the villi level and found that KO males exhibited smaller villi, in terms of both height and thickness, as well as reduced crypt area compared to the WT. These results suggest an overall developmental delay in absence of Cdkl5 and impaired architecture of the intestinal epithelium. Since one of the well-known functions of CDKL5 is the regulation of microtubules (MTs) dynamics through its interaction with IQGAP1, a scaffold protein implicated in MT organization and tight junction (TJ) formation, we analyzed its expression. We found that MDCK cells silenced for CDKL5 present reduced expression of IQGAP1, accompanied by altered morphology, defective paracellular permeability and deregulation of Claudin4. Parallel *in vivo* studies revealed a downregulation of IQGAP1 expression in the mucosal layer. Although preliminary, our intriguing results underline the role of CDKL5 in IEB organization, thus paving the way for a better understanding of GI alterations in CDD and giving novel insights into gut-brain axis regulation.