Travel transforms. Ask Charles Darwin.

When he landed on the Galapagos island of St. Cristobal in 1835, Darwin never imagined he would change humankind forever. The wisdom he gained inspired generations of scientists that came after.

On June 1, 2023, Gastronauts will disembarked on that same St. Cristobal Island to envision the future of knowledge on food, the gut, and the brain.

Gastronauts is the global venue for the dissemination of knowledge on gut-brain matters. In collaboration with USFQ - Ecuador, Galapagos will be the the fourth Global Gastronauts Symposium. It will bring together 80 scientific leaders to imagine the scientific future of how to turn food into precision medicine.

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Gastronauts Galapagos is co-hosted by:

Universidad San Francisco

Agenda

Symposium location is at the Darwin Conference Center (*see map, page 8*) unless otherwise noted.

Day I — June 1, arrival

17:30 - 19:30 **Welcome reception**
Location: USFQ Galapagos Campus (*see map, page 8*)

17:30 - 18:30 Poster Group I
18:30 - 19:30 Poster Group II
** Posters can be set up as early as 17:00*

Day II — June 2

08:00 **Coffee Available**

08:30 - 08:35 **Opening remarks**

08:35 - 09:15 **Keynote:**

Antonio Lazcano — History of Darwin and evolution
UNAM, Mexico

09:15 - 10:45 **Session I: Talking neuro-immune**

John Lukens — Immune-neuron communication
U of Virginia, USA

Gabriel Antonio Trueba — Evolution of microbe gene transfer
U San Francisco de Quito, Ecuador

Asya Rolls — Immunity as a global messenger
Technion, Israel

10:45 - 11:15 **Coffee Break**

11:15 - 12:45 **Session II: Medicinal plants**

Zhen Wang — Metabolic engineering of plants
U of Buffalo, USA

María Elena Cazar-Ramirez — Natural bioagents of Ecuador
U Cuenca, Ecuador

Michael Huffman — The evolution of self-medication
Kyoto U, Japan

12:45 - 16:30 **Lunch Break**

Location: USFQ Galapagos Campus (*see map, page 8*)
** Optional tour of USFQ Science Center (30 minutes)*

16:30 - 18:30 **Session III: Mind-body connection**

Jing Wang — How we detect protein in the diet
U California-San Diego, USA

Teresa Lever — Visualizing digestion
U of Missouri, USA

Corrie Dacosta — Ion channel time travel
U of Ottawa, Canada

Dragana Rogulja — Sensing circadian rhythms
Harvard U, USA

Day III — June 3

08:00 **Coffee Available**

08:30 - 10:30 **Session IV: Biotechnology**

Daniella Oettler *TSE*	Developing intelligent equipment
Yulong Li *Peking U, China*	Seeing the brain in action
Enrique Hernández-Lemus *INMEGEN, Mexico*	Computational genomics
Greg Gage *Backyard Brains, USA*	Democratizing neuroscience

10:30 - 11:00 **Coffee Break**

11:00 - 12:30 **Session V: Gut Physiology**

Gilles Mithieux *U Lyon, France*	Glucose metabolism in evolution
David Hildebrand *Rockefeller U, USA*	A closer look at gut ultrastructure
Frank Reimann *U Cambridge, UK*	How the intestine recognizes food

12:45 - 14:00 **Lunch Break**

Location: USFQ Galapagos Campus (*see map, page 8*)

14:00 - 15:30 **Session VI: Food or Medicine**

Mandë Holford *Hunter College, USA*	Killing pain with venom
Zohar Kerem *Hebrew U of Jerusalem, Israel*	The science of wine
Chefs: Emilio Dalmau and Esteban Tapia *U San Francisco de Quito, Ecuador*	The art of preparing food

Day IV — June 4, departure

Optional breakfast optional departure

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Reference points

1. SCY Airport
2. Miconia hotel

Conference locations

3. Darwin Conference Center
4. USFQ Galapagos Campus

Tourist attractions

5. Interpretation Center: History museum and walking path
6. La Lobería Beach: Snorkeling, 3km/ 7min. taxi from town

Av. de la Armada Nacional
Española
Avenida 12 de Febrero
Avenida Quito
Primera
Avenida Alsacio Northia
Ambato
Alberto Spencer
Av. Jaime Roldós Aguilera
Loberia
Loberia
400km/ 0.25 mile

POSTER PRESENTATIONS:

Day I — June 1, arrival

17:30 - 19:30 POSTER PRESENTATIONS DURING WELCOME RECEPTION

Location: USFQ Galapagos Campus (*see map, page 8*)

17:30 - 18:30 Poster Group I

18:30 - 19:30 Poster Group II

Poster Group I (17:30 - 18:30)	
1	"Hindbrain Glp1r mediate the satiety and weight loss effects of obesity drugs" Alisha Acosta
2	"Stress hormone receptors modify intestinal epithelial signaling" Jacob Allen
3	"Rapid sugar sensing from gut to brain" Emily Alway
4	"Mapping brain-wide responses to gut-mediated signals in larval zebrafish" Minel Arinel
5	"Cognitive Development in Ecuadorian children with PKU" Ana Cacao
6	"Autism spectrum disorders microbiome studies in Ecuador and multi-omic analysis along the gut-brain axis" Paúl Cárdenas
7	"Measuring fetal neuroarchitecture in germ-free mice in response to maternal IL-17A administration" Izan Chalen
8	"Investigation of a novel myenteric neuron class" Yanan Chen
9	"Glutamatergic interneurons regulate intestinal motility" Ryan Hamnett
10	"Eletrophysiological correlates of emotional face processing: Brain responses to ambiguous facial expressions" Natalie Izurieta Hidalgo
11	"Sleep Loss Alters the Gut's Cellular Architecture and Function" Alejandro Laureano
12	"Characterisation of submucosal neuron classes in the mouse small intestine" Wei Li
13	"A sensory neuroepithelial circuit for gut microbial patterns to modulate feeding" Winston Liu
14	"Examining the effect of compounds in food on GLP-1 receptor in the intestine using computational methods"
	"Behavior effects of a blueberry and galacto-oligosaccharide diet on sickness induced deficits in a rodent model" Zohar Kerem

Poster Group II (18:30 - 19:30)	
15	"Neuropod Cell GUCY2C Relieves Visceral Pain" Annie Londregan
16	"Spatial Light Interference Microscopy for Quantification of Brain and Gut Changes in a Murine Repeated Social Defeat Model" Jorge Maldonado
17	"Intelectin-1 control of appetite protects from obesity." Juan Matute
18	"Differential effects of sugars on hunger circuits" Aaron McKnight
19	"The role of mucosal serotonin in visceral nociception" Sarah Najjar
20	"Multi-Parametric Interrogation of the Systemic Lupus Erythematosus (SLE) Immunome Reveals Multiple Derangements" Katherine Nay Yaung
21	"Glucose stimulated GLP-1 and PYY secretion in SGLT1-KO mice is maintained following broad spectrum oral antibiotic treatment" Elisabeth A A O'Flaherty
22	"A brainstem map of visceral sensations" Chen Ran
23	"A gut cell for food pleasure" Laura Rupprecht
24	"Role of the gut microbiome in the development of the enteric nervous system and its link to autism spectrum disorders" Rajlakshmi Sawale
25	"Unveiling the Complexity of Time Discrimination and its Cognitive Implications with a Novel Test: Insights from Behavioral and fMRI Analyses." María Sol Garcés Esponosa
26	"A gut sense for protein" Peter Weng
27	"Characterisation of relaxin/insulin-like family peptide receptor 4 (Rxfp4)-expressing cells in peripheral regions of the gut-brain axis" Orla Woodward
28	"Sexual dimorphism and aging in the development of NASH in mice and the potential use of estradiol supplementation in aged females" Paul Yen

Poster Abstracts:

1. Hindbrain Glp1r mediate the satiety and weight loss effects of obesity drugs

Alisha A Acosta[1], Kuei-Pin Huang[1], Yeno Gbenou[1], and Amber L Alhadeff[1,2]
[1]Monell Chemical Senses Center, Philadelphia PA 19104 USA
[2]University of Pennsylvania, Philadelphia, PA 19104, USA

Long-acting glucagon-like-1 receptor (Glp1r) agonists reduce food intake and body weight in both rodents and humans and are current popular FDA-approved drugs for obesity. Recent studies suggest that these drugs bind Glp1r-expressing neurons that are abundant in the the dorsal vagal complex (DVC), arcuate nucleus (ARC), and vagal afferents [nodose ganglion (NG)]. However, the Glp1r neural population(s) that mediates the therapeutic effects of these obesity drugs is unknown. To determine which population(s) contributes to Glp1r agonist-induced anorexia, we ablated Glp1r-expressing neuron populations within the DVC, ARC, and NG, and measured food intake and body weight after administering Glp1r agonists. Glp1rDVC neuron ablation completely blocked the feeding suppression by exendin-4 (a Glp1r agonist and FDA-approved drug). In contrast, ablation of Glp1rARC and Glp1rNG had no effect on the ability for exendin-4 to suppress feeding when compared to controls. To examine long term effects of glp1r agonists on body weight, diet-induced obese mice with Glp1rDVC neuron ablation were administered semaglutide (the most recent and popular FDA-approved Glp1r agonist) for three weeks. Glp1rDVC neuron ablation prevented weight loss from treatment with semaglutide. Overall these data show that Glp1rDVC neurons are necessary for the anorexic and body weight suppressing effects of obesity drugs.

2. Stress hormone receptors modify intestinal epithelial ROS signaling

Maria Elisa Caetano-Silva[1], Akriti Shrestha[1], Mikaela Webb[1], Chia Hao Lin[1], Jacob M. Allen[1]
[1]Department of Kinesiology and Community Health, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[2]Center for Microbial Pathogenesis, Nationwide Children's Hospital, Columbus, OH, USA

Psychological stress increases risk of inflammatory bowel disease (IBD). Intestinal epithelial cells (IECs) maintain homeostasis between the gut microbiota and its host and have been implicated in IBD. We previously reported stress-induced IEC reactive oxygen/nitrogen species (ROS/RNS) signaling – which we hypothesize to underly mucosal dysfunction and IBD predisposition. Here, we examined the role of sympathetic nervous system in mediating IEC ROS/RNS signaling through pharmacological blockade α and β-adrenergic receptors (AR) in mice exposed to social disruption stress (SDR). Adult male C57BL/6 mice (n=6/group) were injected i.p. daily with 1) saline 2) α2-(Idazoxan-2mg/kg) or 3) β-AR antagonists (Propranolol-10mg/kg) during SDR for 6 days. Colonic IECs were isolated ($CD45^-$; $EpCAM^+$) and analyzed for expression of ROS/RNS related enzymes by qPCR. Stress upregulated dual-oxidase 2 (*Duox2*) and inducible nitric oxide synthase *(Nos2*) expression in IECs ($p<0.05$), indicating heightened ROS/RNS signaling. Blockade of α-AR did not impact the stress-induced *Duox2* or *Nos2* expression. IECs lack β-AR, yet *in vivo* blockade of β-AR completely inhibited stress-induced upregulation of *Nos2,* but not *Duox2,* (*Nos2*-Stress x propranolol $p<0.05$). We suspect *Nos2* expression is regulated by β-AR activation mechanisms extrinsic to IECs. Ongoing experiments are examining glucocorticoid signaling as a mediator of stress-induced IEC-ROS signaling. Supported by NIH R01DK131133-0.

3. Rapid sugar sensing from gut to brain

Emily J. Alway[1], Peter J. Weng[1], Laura E. Rupprecht[2], Winston W. Liu[1], M. Maya Kaelberer[2], & Diego V. Bohórquez[1,2]
[1]Department of Neurobiology
[2]Division of Gastroenterology, Department of Medicine, Duke University School of Medicine

The average American adult consumes over 40 pounds of sugar per year. While sugar intake is necessary for energy metabolism and survival, this overconsumption has led to rampant obesity and diabetes. Therefore, it is critical to determine the circuits that drive sugar overconsumption, including gut-brain circuits. Recently, specialized sensory cells in the intestinal epithelium, known as neuropod cells, were found to sense intestinal sugars and drive sugar appetite. Neuropod cells sense sugars using sodium-glucose transporters (SGLTs). Most studies on intestinal sugar sensing have focused on glucose transporter SGLT1 but little is known about SGLT3, a sugar sensor with no transport activity. Here, we use RT-qPCR and fluorescence in situ hybridization to determine expression of glucose transporters and sensors in neuropod cells. We additionally test whether the anti-diabetic molecule and SGLT3 agonist miglitol activates neuropod cells. Finally, we test whether miglitol infusion into the duodenum causes vagal activity. We find that SGLT3 transcripts are upregulated in neuropod cells relative to other intestinal epithelial cells. Additionally, while SGLT3 agonist miglitol activates neuropod cells, intestinal miglitol does not cause vagus nerve activity. These studies may uncover a pharmacological target for modulating rapid gut-brain control of food choice without perturbing life-sustaining sugar absorption.

4. Mapping brain-wide responses to gut-mediated signals in larval zebrafish

Minel Arinel, Matthew D. Loring, Evan P. Drage, Eva A. Naumann
Department of Neurobiology, Duke University

Specialized sensory cells in the gut epithelium termed enteroendocrine cells (EECs) have been shown to form synaptic connections with vagal sensory neurons, sending enteric information directly to the brain through the vagus nerve. Yet, we lack basic conceptual insights into the way gut-brain circuits encode sensory information to impact neural activity across the brain. The translucent zebrafish is an ideal model due to its optical accessibility across the entire gut-brain circuitry at single-cell resolution. Here, we demonstrate a method to study the effects of EEC-mediated signals on vagal and brain-wide activity using chemical and optogenetic approaches in larval zebrafish. To map functional responses, we engineered a computer controlled microgavage system to inject nanoliter volumes of distinct stimuli directly into the intestinal lumen of larval zebrafish while performing calcium imaging via volumetric two-photon microscopy. Using precisely timed injections of EEC-activating nutrients directly into the intestinal bulb, we show differential activation of vagal and hindbrain neurons in fish with different feeding experiences, suggesting specific enteric sensory encoding across these brain areas. Finally, optogenetic photostimulation of EECs along the gut directly implicates these cells in driving neural activity and establishes topographic representations of the gut across the brain.

5. Cognitive Development in Ecuadorian children with PKU

Ana Paula Cacao[1], Karen Larrea[2], Priscila Navarrete[2], Vanessa Romero[3], Valeria Troya[2], Nergiz Turgut[2,4]
[1]Colegio de Ciencias Biológicas y Ambientales
[2]Colegia de Ciencias Sociales y Humanidades

[3]Colegio de Ciencias de la Salud
[4]Instituto de Neurociencias Universidad San Francisco de Quito

This study investigates the relationship between phenylketonuria (PKU) and cognitive impairments in Ecuadorian children. PKU is an inborn error in metabolism caused by variants of the PAH gene. Hyperphenylalaninemia, the core biochemical abnormality in PKU, leads to malfunction in cerebral protein and neurotransmission synthesis. A strict low-Phenylalanine diet has proven to be very effective for preventing the impaired cognitive development associated with the disorder. PKU is a degenerative disease where late diagnosis can lead to neurodevelopmental impairments. However, it is not entirely clear which cognitive areas are most affected. Given the lack of postnatal screening in Ecuador, PKU is often diagnosed after several years. The following study has the objective to investigate the cognitive status in ecuadorian children with PKU. Intelligence Quotient and executive functions will be assessed in approximately 20 children in the area of Quito and Salcedo (i.e. 3-16) in May 2023 by the implementation of WPPSI- IV, WISC-V and NEPSY-II (Preliminary data will be available in June). Hence, this study has the potential to provide more insight to the possible impairments related to PKU and for future studies to develop treatment and psychoeducation programs.

6. Autism spectrum disorders microbiome studies in Ecuador and multi-omic analysis along the gut-brain axis

Andrés Suarez1, Marco Fornasini2, Maria Fernanda Zurita1, James T. Morton3, Gaspar Taroncher-Oldenburg[3], Manuel E. Baldeón[2], Paúl A. Cárdenas[1]
1-Instituto de Microbiología, Universidad San Francisco de Quito
2-Facultad de Medicina, Universidad Internacional del Ecuador
3-Center for Computational Biology, Flatiron Institute, Simons Foundation, New York, USA

Disruption of the gut-brain axis (GBA) has been implicated in autism. To explore the functional architecture of autism, we developed an age and sex-matched Bayesian differential ranking algorithm that identified autism-specific profiles across 10 cross-sectional microbiome datasets and 15 other omic datasets, including dietary patterns, metabolomics, cytokine profiles, and human brain expression profiles. This architecture was determined by autism-specific amino acid, carbohydrate and lipid metabolism profiles predominantly encoded by microbial species in the genera Prevotella, Enterococcus, Bifidobacterium, and Desulfovibrio, and was mirrored in brain-associated gene expression profiles and restrictive dietary patterns in individuals with autism. Pro-inflammatory cytokine profiling and virome association analysis further supported the existence of an autism-specific architecture associated with particular microbial genera. Further elucidation of the functional architecture of autism, including of the role the microbiome plays in it, will require deep, multi-omic longitudinal intervention studies on well-defined stratified cohorts to support causal and mechanistic inference.

7. Measuring fetal neuroarchitecture in germ-free mice in response to maternal IL-17A administration.

Izan Chalen, Selena Wang, Catherine Best-Popescu, and Adrienne Antonson
University of Illinois Urbana-Champaign

Infection-induced maternal immune activation (MIA) during pregnancy has been linked to neurodevelopmental disorders in the offspring. Researchers have suggested strong links between fetal

cortical malformations and the maternal interleukin (IL)-17A pathway, which in turn is linked to the maternal microbiota. Whether effector cytokine IL-17A is sufficient for causing fetal brain abnormalities in the absence of microbes, however, has not been determined. In this study, pregnant germ-free mice were sterilely injected with either recombinant mouse IL-17A or vehicle daily during gestational day (GD)10.5 through GD15.4, with sacrifice performed on GD16.5. Fetal brains were sectioned coronally and immunohistochemically stained for cortical layer development and structure. Tissues were imaged using traditional fluorescence microscopy and Spatial Light Interference Microscopy. Mapping of cortical layer development and structure, via expression of neuronal transcription factors, is ongoing. Through this project, we aim to disentangle the effects of IL-17A from those of maternal microbes on fetal neocortical development.

8. Investigation of a novel myenteric neuron class

Yanan Chen[1], Anastassia Mikhailova[1], Wei Li[1], Khomgrit Morarach[1], Arthur Beyder[2], David R. Linden[3] and Ulrika Marklund[1]
[1]Division of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden
[2]Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, US
[3]Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, US

The gastrointestinal tract is regulated by the enteric nervous system, consisting of numerous neuron subtypes that work in concert to achieve complex functions such as the peristaltic movements. However, the full diversity among enteric neurons and their dedicated functions are still not resolved. Recently, our lab utilized single-cell RNA sequencing to molecularly define myenteric neuron types (Morarach et al., 2021). A novel subtype, ENC*7* that specifically expresses cholecystokinin (*Cck*) and urocortin 3 (*Ucn-3*), was computationally defined as intrinsic primary afferent neurons (IPANs) due to its expression of previously defined IPAN markers. Nevertheless, its morphology indicating that ENC*7* is not a classical IPAN. To predict its connectivity patterns in the gut wall, we injected *Cck-Ires-Cre* mice with a novel AAV-type virus containing a Cre-dependent fluorescent reporter. Plausible interacting cells were visualized by simultaneous detection of histochemical marker genes. Analysis of ENC*7* boutons in association with cell bodies indicated that ENC*7* selectively communicate with certain neuron classes. Intriguingly, connection between enteric neurons and pancreas were observed, indicating a direct neuronal route between gut and pancreas. This study will not only contributing to a better understanding of ENS functions, but also novel insights of relevance for gut-brain axis which may be of clinical interest.

9. Glutamatergic interneurons regulate intestinal motility

Ryan Hamnett
Stanford University

The enteric nervous system (ENS) is situated in the intestinal wall and autonomously controls most aspects of digestion, including intestinal motility. The current model of neuronal control of intestinal motility describes reflex circuits responding to locally detected luminal contents, but provides no explanation for long-distance communication and organ-wide responses to stimuli. However, like the central nervous system, the ENS contains a diverse array of neuronal subtypes, and the contributions of many of these subtypes to intestinal function are unknown. The excitatory neurotransmitter glutamate is expressed in a small number of enteric neuron subtypes, the characteristics, circuitry and function of which have been little explored, though dysregulation of glutamatergic signalling is

thought to be involved in several pathologies of the digestive tract, including inflammatory bowel disease and ischemia/reperfusion injury. We use optogenetic activation, immunohistochemistry and single neuron tracing of glutamatergic neurons to demonstrate their role in colonic motility. Additionally, we identify a novel neuron subpopulation that expresses glutamate and is present only in the colon. This is the first illustration of glutamatergic neuron function in the gut, and could represent a mechanistic basis for long-distance communication in the ENS.

10. Electrophysiological correlates of emotional face processing: Brain responses to ambiguous facial expressions

Natalie A. Izurieta Hidalgo
Universidad San Francisco de Quito

Due to the evolutionary significance of emotions, the information faces offer about the emotional states of others should be processed quickly to help the online regulation of behaviours. Several neuronal systems are involved such the occipital cortices, the amygdala, the orbitofrontal cortex, the basal ganglia, as well as the right parietal cortex. The processing of emotional faces consists of an initial rapid detection of facial expression, followed by an extended attentive processing. Electroencephalography-based event-related potential (ERP) technique allows examining brain responses to specific stimuli providing a high temporal resolution and a non-invasive online registration. Biases and alterations in facial emotion processing have been studied across many psychiatric conditions. In borderline personality disorder (BPD), our findings suggest reduced thresholds for facial anger and deficits in the discrimination of positive facial expressions. The current data could help to explain the negative perception of others that may be related to the characteristic impairment in interpersonal functioning in BPD.

11. Sleep Loss Alters the Gut's Cellular Architecture and Function

Alejandra Laureando
Harvard University

We discovered that sleep loss leads to accumulation of reactive oxygen species (ROS) in the gut, with gut oxidation explaining why severe sleep loss can result in premature death. Our new data show that in addition to causing oxidative stress, accumulated ROS molecules cause restructuring of the gut on a cellular level. During sleep restriction, cell proliferation is promoted in a specific area of the gut in a ROS-dependent manner, and the reorganized tissue becomes enriched in enteroendocrine cells, cells that resemble neurons and secrete various neuropeptides. I will show evidence that some of the enteroendocrine cells signal from the gut of sleep-deprived animals to the brain, to change the quality of sleep (i.e. to increase the depth of sleep in animals which sleep little). Both the changes in gut composition, and in sleep quality persist for a while after sleep loss is stopped. My goal is to determine how the changes in the gut impact the function of this organ in regulating sleep, and possibly other behaviors or physiological processes, when sleep is restricted. Doing so could be transformative for understanding how sleep problems affect health and designing potential solutions to reverse the damage caused by poor sleep.

12. Characterisation of submucosal neuron classes in the mouse small intestine

Wei Li, Khomgrit Morarach, Anastassia Mikhailova and Ulrika Marklund

Department of Medical Biochemistry and Biophysics, Unit of Molecular Neurobiology, Karolinska Institutet, Stockholm, Sweden.

The gastrointestinal tract's intrinsic nervous system, named the enteric nervous system, controls different aspects of the gut's physiology including muscle movement, blood flow and secretion. These functions are implemented through the combined activities of different molecularly distinct neuronal types. We have previously uncovered molecularly distinct myenteric neurons in the mouse small intestine via single-cell RNA sequencing (Morarach *et al.*, 2021). Here, we define three cardinal submucosal neuron classes (smENC1-3) in the aforesaid organ, which correspond to the presumed cholinergic secretomotor (*Sst*$^{+}$, smENC2) and noncholinergic secretomotor/vasodilatory neurons (*Vip*$^{+}$, smENC3). Additionally, we find evidence for a submucosal sensory neuron class in the former (*Nmu*$^{+}$, smENC1).

We report single-cell labeling of each neuron class by systemic injection of AAV.PHP.S-DIO-EFYP on Cre-driver mouse lines to study their morphology. smENC1 possesses a smooth soma with several long processes, whereas smENC2 and smENC3 display short lamellar dendrites and one long process. Furthermore, we investigated the connectivity between smENCs by simultaneous immunohistochemical labeling of target neurons. Our preliminary observations indicate substantial self-connectivity as well as connections between classes.

Our study provides comprehensive insight into submucosal enteric neurons from their molecular signatures to their morphology and the potential circuits they form.

13. A sensory neuroepithelial circuit for gut microbial patterns to modulate feeding

Winston W. Liu[1,2], Chloe Schaefgen[3,4], Laura E. Rupprecht[1,2], Peter Weng[1,3], Jorge Villalobos[2], M. Maya Kaelberer[1,2], and Diego V. Bohórquez[1,2,4]
[1]Department of Neurobiology, Duke University
[2]Department of Medicine, Duke University
[3]Trinity College of Arts & Sciences, Duke University
[4]Duke Institute for Brain Sciences, Duke University

Gut microbes and their host need to eat, but microbes rely on their host to access nutrients. Thus, microbes use microbial patterns and metabolites to influence the appetite of its host, including the quantity, quality, and timing of food intake. But the specific molecules, cells, transmitters, and circuits used by the host to sense and respond to the luminal stimuli of microbial patterns in real time remain unknown. In the small intestine, nutrients guide appetitive choices by eliciting fast sensory signals through neural circuits beginning with intestinal neuropod cells. Here, we show that in the colon, neuropod cells labeled by peptide YY (PYY) transduce stimuli from the microbial pattern flagellin onto the vagus nerve to alter feeding behavior using the microbial pattern recognition receptor toll like receptor 5 (TLR5). Rather than directly activating vagal neurons, colonic flagellin reduces food intake by stimulating PYY cells in a manner independent of inflammation. The transduction of flagellin stimuli depends on the release of the neuromodulator PYY that, in turn, activates a metabotropic receptor on vagal neurons. Our results reveal a novel sensory modality, distinct from the immune system, that enables the host to adjust its appetitive behavior by detecting microbial patterns in a discrete location of the gastrointestinal tract.

14(a). Examining the effect of compounds in food on GLP-1 receptor in the intestine using computational methods

Ayelet Bait Halachmy, Adi Nudel, Zohar Kerem
Robert H. Smith Faculty of Agriculture, Food and Environment,
The Hebrew University of Jerusalem, Israel

Machine learning (ML) has revolutionized computational biology and computational chemistry, transforming our understanding of molecular structure and chemical properties. Glucagon-Like Peptide 1 (GLP-1) is a hormone that regulates glucose metabolism and appetite by binding to its receptor GLP-1R. Computational approaches served to predict and identify small molecules from food that selectively bind and either activate or inhibit GLP-1R. GLP-1 is produced by L cells in the intestine in response to nutrients. Docking techniques and computational models have been developed to detect food compounds similar to known GLP-1 receptor agonists. However, current docking models have limitations in scanning large databases due to accuracy and runtime issues. Nonetheless, promising small molecule candidates identified through computational methods were validated in vitro, showing effects on insulin secretion in mouse beta cells. This highlights the potential of computational approaches in discovering compounds that modulate GLP-1R activity and impact glucose metabolism and appetite regulation.

14(b). Behavior effects of a blueberry and galacto-oligosaccharide diet on sickness induced deficits in a rodent model

Jennifer S Jordan, Richard S. Bruno, Cole Vonder Haar & Yael Vodovotz
* Presented by Zohar Kerem
Department of Food Science and Technology,The Ohio State University
Email: vodovotz.1@osu.edu

Blueberries and Galacto-oligosaccharides (GOS), a prebiotic, modulate the gut microbiome, while the anti-inflammatory properties of anthocyanins (ACN) have been shown to improve memory in rats. This study aimed to investigate the effects of a diet consisting of blueberries and GOS on sickness-induced rodents, specifically examining sickness effects, depressive-related behavior, anxiety-like behavior, and learning.

The findings indicate that both diets influenced rodent weight, with no significant effect observed on depressive-related behavior. However, the administration of lipopolysaccharide (LPS) increased anxiety-like behavior. The inclusion of blueberries and GOS in the diet appeared to mitigate the anxiety caused by LPS. Moreover, neither LPS nor the diet had a significant impact on learning acquisition in the Morris Water Maze. However, LPS did significantly affect working memory during repeat acquisition in the Morris Water Maze on day 2.

Next we plan to connect behavior results with physiological differences, and perform an Acute Study on the interaction between diet and LPS Clinical trial.

15. Neuropod Cell GUCY2C Relieves Visceral Pain

Annie K Londregan[1,*], Joshua R Barton[1,*], Tyler Alexander[2,*], Adam E Snook[1], Manuel Covarrubias[2,+], Scott A Waldman[1,+]
[1]Pharmacology, Physiology, and Cancer Biology
[2]Vickie and Jack Farber Institute for Neuroscience, Thomas Jefferson University, Philadelphia, PA

*, +Authors contributed equally

Guanylyl cyclase C (GUCY2C), a membrane receptor expressed by all intestinal epithelial cells, produces cyclic (c)GMP to regulate luminal fluid secretion. This mechanism has been leveraged to develop GUCY2C agonists, including the FDA-approved drug linaclotide which is used to relieve irritable bowel syndrome with constipation (IBS-C). Beyond constipation, linaclotide also relieves visceral pain associated with IBS in mice and humans, although mechanisms remain incompletely understood. Recently, we identified a novel population of rare intestinal cells that over-express GUCY2C (GUCY2CHigh), with phenotypic and transcriptomic characteristics of the newly identified neuropod cell, a subset of enteroendocrine cells concentrated in small intestine. In co-cultures, GUCY2CHigh neuropod cells synapse with, and spontaneously excite, dorsal root ganglion neurons (DRGs). Notably, linaclotide reduces this excitability in DRGs synapsing with neuropod cells from wild type, but not GUCY2C-knockout, mice; this effect is not reproduced by extracellular cGMP. In vivo, eliminating GUCY2C expression selectively in neuropod, but not other intestinal epithelial, cells produced a spontaneous visceral pain syndrome that was refractory to linaclotide. These data suggest that GUCY2C specifically in neuropod cells mediates visceral analgesia produced by linaclotide.

16. Spatial Light Interference Microscopy for Quantification of Brain and Gut Changes in a Murine Repeated Social Defeat Model

Jorge Maldonado
University of Illinois Urbana-Champaign

We use Spatial Light Interference Microscopy (SLIM), a label-free technique for advanced digital histopathology of brain sections to quantify inflammation and cellularity in mice subjected to repeated social defeat (RSD). Gastrointestinal structural alterations in the RSD mice were compared to treated and normal control animals. RSD mice were treated with anti-corticosteroid, beta blocker, or selective serotonin reuptake inhibitor (SSRI), to explore the effects of these drugs on brain cyto and myeloarchitecture and inflammation in RSD mice.

We measured brain structural changes and inflammatory parameters in the amygdala, cortex, hippocampus, cerebellum, corpus collosum, perivascular and periventricular regions. The results show that all treatments show differences in brain and gut structure and inflammatory parameters compared to control group. The findings suggest that these drugs may decrease brain structural changes and reduce inflammation in RSD models. SLIM imaging was demonstrated as a valuable tool for quantifying brain structural changes and inflammatory parameters with high sensitivity in RSD models. This technique has the potential to significantly advance our understanding of the effects of chronic stress and the influence of chronic social stress in brain inflammation and gut permeability, and to evaluate new therapeutic targets for treating stress-related disorders.

17. Intelectin-1 control of appetite protects from obesity

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Single nucleotide polymorphisms (SNPs) in Intelectin-1 are associated with obesity, and Intelectin-1 is decreased in patients with obesity. Intelectin-1 is a lectin secreted by intestinal epithelial cells. ITLN1 binds exclusively to bacterial glycans. We used Itln1$^{-/-}$ mice to evaluate Intelectin-1 potential role in obesity pathogenesis. Intelectin-1 was only detected in the intestinal lumen of mice. In diet-induced obesity, Itln1$^{-/-}$ mice developed worse obesity than wt littermates after exposure to high-fat diet at weaning. In comprehensive metabolic assessment, Itln1$^{-/-}$ mice had increased cumulative food intake and positive energy balance with no differences in energy expenditure, locomotor activity, or energy content of stools compared to wt mice. Lastly, depletion of the microbiota with broad-spectrum oral antibiotics mitigated obesity development in Itln1$^{-/-}$ mice. Our finding suggests that luminal Intelectin-1 in the intestine protects from obesity by regulating appetite perhaps through a microbiota-mediated mechanism. We are currently exploring potential mechanisms mediating the observed phenotype.

18. Differential effects of sugars on hunger circuits

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Activity in hypothalamic hunger circuits drives feeding behavior. Our recent work demonstrated that sugar and fat inhibit neural activity in hypothalamic agouti-related protein (AgRP)-expressing neurons via distinct neural gut-brain mechanisms. Here, we examined whether different sugars, glucose and fructose, differentially impact activity in AgRP neurons. Using fiber photometry, we monitored in vivo AgRP neural activity in mice outfitted with intestinal catheters. We found that equicaloric intestinal infusions of fructose were significantly less effective than glucose at inhibiting AgRP neuron activity. We also discovered that the time to maximally inhibit AgRP activity was slower after fructose infusion compared to glucose infusion, suggesting that fructose may modulate hunger circuits via an endocrine/paracrine mechanism rather than through a neural circuit. We therefore investigated the role of humoral signaling in the fructose-mediated inhibition of AgRP neuron activity. Pretreatment with a Y2 receptor antagonist (the receptor for the satiation signal Peptide YY) attenuated fructose-induced inhibition of AgRP neuron activity, whereas pretreatment with antagonists for other satiation signal receptors (CCKAR, 5-HT3R, GLP1R, or CALCR) had no effect. Overall, these data show that fructose is less effective as glucose at modulating AgRP activity, and that Y2 receptor signaling is involved in the fructose-mediated inhibition of AgRP activity.

19. The role of mucosal serotonin in visceral nociception

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Irritable bowel syndrome (IBS) is a prevalent disorder characterized by visceral pain and dysmotility. Serotonin (5-HT) contributes to visceral pain signaling, but the efficacy of targeting 5-HT to treat IBS is limited and fraught with adverse effects. Enterochromaffin (EC) cells in the gastrointestinal epithelium produce most of the 5-HT in the gut, which stimulates extrinsic primary afferent neuron (ExPAN) terminals to promote sensory signaling. The serotonin reuptake transporter (SERT), present throughout epithelial cells, rapidly inactivates 5-HT. This study tested the hypotheses that 1) 5-HT released from EC cells and 2) SERT-mediated regulation of mucosal 5-HT availability modulate visceral nociception. Mice with a lack of mucosal 5-HT (TPH1 KO or peripheral TPH inhibitor) or

excess of mucosal 5-HT (SERT-Floxed/Villin-cre or SERT-Floxed/Villin-creERT2) underwent visceral nociceptive testing. TPH1 KO mice and TPH inhibitor-treated mice displayed a sex-dependent decrease in visceral nociception and SERT-Floxed/Villin-cre mice displayed a sex-dependent increase in visceral nociception. The sex differences observed in these studies indicate that treatments for visceral pain that are targeted to serotonin signaling may have different efficacies in males vs. females. Future studies will investigate the underlying causes of these sex differences and will test the effects of acute activation/inhibition of EC cells on visceral pain.

20. Multi-Parametric Interrogation of the Systemic Lupus Erythematosus (SLE) Immunome Reveals Multiple Derangements

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease with unpredictable disease course. To gain mechanistic insights for better clinical assessment, SLE is best interrogated with a multi-parametric, holistic approach such as mass cytometry (CyTOF). Peripheral blood mononuclear cells from 26 patients (41 samples, median age 39.5years) and 27 age-matched subjects underwent CyTOF using 43 immune markers. Analysis was performed using an immunomics machine learning platform. Numerous derangements were seen in SLE, with significant changes in the $FoxP3^{+}CTLA4^{+}CD4^{+}$ T cell clusters. These consist of both CD45RA+(memory) and CD45RA-(naïve) natural regulatory T cells (T_{REG}s) that express CD25 and T_{REG}-like cells that do not. Natural TREGs are unchanged, indicating absence of a compensatory, regulatory-driven response to disease inflammation. However, T_{REG}-like cells ($CTLA4^{+}TIGIT^{+}CLA^{+/-}PD1^{+/-}$) are significantly increased in disease, suggesting a deranged immunoregulatory response, since CTLA4, PD1 and TIGIT are cell checkpoint inhibitors in normal physiology. There was also significant reduction in CTLA4 expression in $CD45RA^{+}$ and $CD45RA^{-}$ $CD4^{+}$ T cells in disease. CTLA4 reduction can potentially lower the immunogenic signal required to elicit an immune response. Therefore, the reduction may be associated with a pro-inflammatory intracellular signaling milieu in disease. With a multi-parametric unbiased approach, we identified several immune subsets of immunopathogenic importance for further functional studies.

21. Glucose stimulated GLP-1 and PYY secretion in SGLT1-KO mice is maintained following broad spectrum oral antibiotic treatment

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SGLT1 facilitates postprandial intestinal glucose absorption and is needed for ex vivo glucose stimulated GLP-1 and GIP secretion from L- and K-type enteroendocrine cells. In contrast, SGLT1 inhibition or KO in vivo is associated with exaggerated glucose stimulated GLP-1 levels, without any accompanying GIP secretion. Here, we explored the potential role of gut microbiota in this yet unexplained glucose stimulation of GLP-1 secretion in SGLT1-KO mice.

SGLT1-WT, but not SGLT1-KO, intestinal organoids secreted GLP-1 in response to D-Glucose. Organoids did not secrete GLP-1 in response to L-Glucose (a "non-metabolisable" D-Glucose enantiomer, not a SGLT1 substrate). In SGLT1-WT, but not SGLT1-KO mice, plasma tGIP increased following oral D-Glucose. SGLT1-WT mice increased plasma tGLP-1, but not PYY, at 5mins following oral D-Glucose. At 60mins, D-Glucose stimulated tGLP-1 returned to baseline in SGLT1-WT mice, while plasma tGLP-1 and PYY were elevated in SGLT1-KO. L-Glucose increased 60mins plasma tGLP-1 and PYY in SGLT1-WT and SGLT1-KO mice, without increasing tGIP.

Antibiotic treatment of SGLT1-WT and SGLT1-KO mice increased basal plasma tGIP, tGLP-1 and PYY levels but did not affect D-Glucose and L-Glucose stimulated hormone secretion patterns. Our results suggest glucose-stimulated GLP-1 and PYY release in SGLT1-KO mice is not dependent on gut microbiota.

22. A brainstem map of visceral sensations

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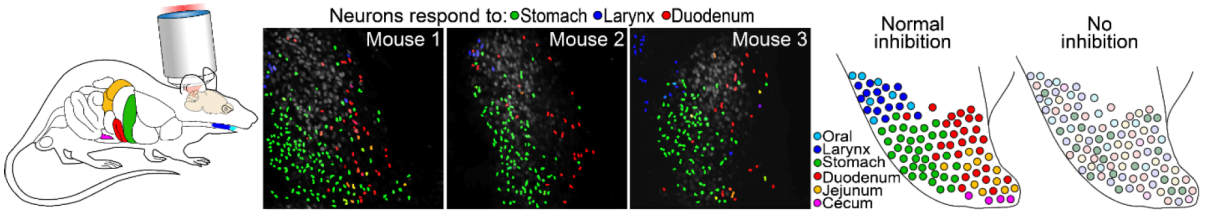
The discoveries of the coding principles of vision, somatosensation, olfaction, gustation, and audition are all landmark achievements. By contrast, little is known about how the brain encodes information from the internal organs to generate our visceral senses. Here we developed an in vivo two-photon mouse brainstem calcium imaging preparation to study the representations of internal organs in the nucleus of the solitary tract (NTS), the brain's viscerosensory gateway. We discovered that the NTS uses a combinatorial code to represent diverse mechanical and chemical stimuli within the same organ. By contrast, different organs are represented by discrete and selectively tuned neuronal ensembles, each comprised of heterogeneous cell types. Organ representations in the brainstem are topographically organized and take the shape of a homunculus. Spatial organization of different organs is further sharpened by inhibition, as blockade of NTS inhibition broadens neural tuning and blurs visceral representations. These studies reveal basic coding principles used by the brain to process visceral inputs (Ran et al., Nature, 2022).



23. A gut cell for food pleasure

Laura Rupprecht
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Guided by gut sensory stimuli, animals prefer foods rich in fats and sugars, but how to gut signals such preferences remained unknown. In the intestinal epithelium, neuropod cells synapse with vagal neurons to convey nutrient stimuli to the brain within seconds. Using optogenetics inside the gut lumen, we discovered that neuropod cells differentiate and transduce luminal stimuli from sweeteners and sugars to drive sugar preference. Moreover, stimulating neuropod cells using optogenetics stimulates a real-time preference for a place. These preferences depend on the neurotransmitter glutamate, which is required for fat and sugar stimuli to stimulate neural activity in the vagus nerve. Neuropod cells in the proximal intestine guide mice to choose foods that evoke a pleasurable feeling.

24. Role of the gut microbiome in the development of the enteric nervous system and its link to autism spectrum disorders

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The gut is lined by a complex network of neurons, the enteric nervous system (ENS), which regulates gut homeostasis, local immune responses, and the microbiome. Gastrointestinal disorders are a common comorbidity associated with neurological disorders such as autism spectrum disorder. However, the mechanistic links remain elusive, so we are using the zebrafish animal models to study the gut-brain axis in vivo and manipulate it in-situ. We hypothesize that the ENS is also affected in neurological disorders, which would explain the common gastrointestinal disorders that accompany such disorders. We are studying the early development of the ENS and how it is modulated by the gut microbiome in wildtype zebrafish larvae and zebrafish models of autism using light sheet microscopy. The ENS activity increases through development, whereas in germ-free animals, it is perturbed and seems to develop faster. We also observed immunological differences with a significant increase in the number of microglia in valproic acid treated larvae (a chemical model of autism) compared to wildtypes. This study thus explores the delicate relationship between the gut microbiome and neuro-immune function of the ENS, and how its dysregulation affects development and disease.

25. Unveiling the Complexity of Time Discrimination and its Cognitive Implications with a Novel Test: Insights from Behavioral and fMRI Analyses.

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Temporal perception is a crucial component of cognitive functioning, and its impairment is a primary cognitive deficit in schizophrenia. FMRI studies have shown that brain networks responsible for time processing are also activated during other cognitive processes that require varying levels of cognitive effort. In this study, we developed a test incorporating tasks from diverse paradigms, including temporal discrimination, saliency, and cognitive control. The efficacy and performance of the test were evaluated, and subsequent fMRI analysis was conducted to investigate brain activity patterns associated with paradigms. The results indicated that the stimuli enabled subjects to detect changes at different difficulty levels. Our findings are consistent with prior research on the activation patterns associated with temporal discrimination and oddball tasks, suggesting that timing circuits may play a role in more complex cognitive processes beyond time perception. The study establishes the

methodology's validity for a larger population, particularly for investigating neuropsychiatric conditions.

26. A Gut Sense for Protein

Peter Weng
Duke University

From single cells to entire animals, amino acids are essential for survival. However, how an animal coordinates the detection of this essential nutrient remains unclear. Dietary amino acids are made available to an animal only after digestion within the small intestine. As such, the first opportunity to evaluate diets for their amino acid content is in the intestine.
In the lining of the intestinal epithelium, neuropod cells transduce nutrient signals to guide nutrient preferences. Here, we demonstrate that following amino acid depletion, STC-1 cells (a gut sensory cell line) enhance detection of specific amino acids and increase expression of certain transcription factors, transporters, and surface receptors. In mice, dietary depletion of amino acids increases a mouse's preference for amino acids in a matter of days. Moreover, in mouse intestinal neuropod cells, dietary depletion of amino acids alters the expression of transcripts in a similar fashion to STC-1 cells. This work establishes the basis for a mechanism in which sensory cells of the gut may direct a mouse's preference for amino acids.

27. Characterisation of relaxin/insulin-like family peptide receptor 4 (*Rxfp4*)-expressing cells in peripheral regions of the gut-brain axis

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Relaxin/insulin-like family peptide receptor 4 (RXFP4) is the receptor for the microbially regulated hormone insulin-like peptide 5 (INSL5), secreted from enteroendocrine L-cells in the distal gastrointestinal tract. INSL5/RXFP4 have recently been implicated in gut motility and feeding regulation as chemogenetic manipulation of *Insl5*- and *Rxfp4*-expressing cells modulates colonic propulsion and food intake in mice. Here, we implemented an *Rxfp4*-Cre reporter mouse as a functional tool to characterise *Rxfp4*-expressing cells in peripheral regions of the gut-brain axis to further explore INSL5/RXFP4 physiology. Using transcriptomic techniques such as qRT-PCR, RNAscope and RNA sequencing, *Rxfp4* expression was identified in enterochromaffin, L and tuft cells in the colonic epithelium and in dorsal root ganglia (DRG) and nodose ganglia (NG) cells of the peripheral nervous system that connect the gut to the brain. By combining *Rxfp4*-Cre mice with a cyclic adenosine monophosphate (cAMP) imaging technique, INSL5 was found to reduce intracellular cAMP levels in *Rxfp4*-expressing cells from a colonic organoid model and in primary cultures of DRG and NG, and thus may modulate hormone and neurotransmitter release from these cells. Further research *in vivo* is required to establish whether INSL5/RXFP4 signalling in these peripheral gut-brain regions underlie the gut motility and feeding phenotypes previously observed.

28. Age and sex disparity in NASH and the potential role of estradiol supplementation for NASH

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Introduction: NAFLD and NASH exhibit higher prevalence in men than women during their reproductive ages but have similar prevalence after menopause. Reproductive age women with NASH have less hepatic fibrosis han men but lose this protection after menopause. TThe underlying mechanism(s) for this sex disparity is not fully understood but may be due to differences in circulating estrogen levels. We thus conducted animal experiments to investigate: 1) sex differences in the histiology, inflammation, and fibrosis in young vs. old male and female mice and 2) the effects of estradiol administration on these parameters in old female mice to evaluate the therapeutic potential of estradiol in NASH.

Methodology: Young/reproductive age (12-week age) and old/post-reproductive age female and male (48~50-week age) C57BL/6J mice were fed control chow diet or high-fat methionine/choline-deficient diet for six weeks to induce NASH. The old mice were administered 17β-estradiol at two doses, 200nM (physiological) or 1000nM (supraphysiological) via drinking water. We analyzed serum and hepatic NASH parameters, ER stress, and lysosome-autophagy defects.

Results and conclusion: Among young mice with NASH, females had less histologic evidence of inflammation and fibrosis, decreased gene expression of Il1b, Il6, Tgfb, Col1a1, Ccl2, and Ccl5, and lower hepatic collagen levels than males. Young female mice with NASH also had less inhibition of hepatic autophagy and lysosomal protein expression than young males with NASH. Both old female and male mice showed NASH progression with age. However, old female mice had more severe hepatic inflammation and fibrosis and higher expression of inflammation and fibrosis genes, and comparable autophagic inhibition and lysosomal defects as similar age males. Physiological doses of estradiol significantly reduced hepatic gene expression of inflammatory and fibrogenic markers, ER stress, and lysosomal-autophagic defects in old females. In contrast, supraphysiological doses of estradiol significantly increased gene expression of inflammation and fibrogenic markers in old females with NASH. In conclusion, similar to clinical NASH, we observed marked sex and age-related differences in the severity of NASH mice. Furthermore, physiological doses of estradiol treatment improved the hepatic histology, inflammation, fibrosis, and lysosome-autophagy defects in post-reproductive age female mice with NASH. These findings suggest that estrogen therapy may benefit post-menopausal patients with NASH.