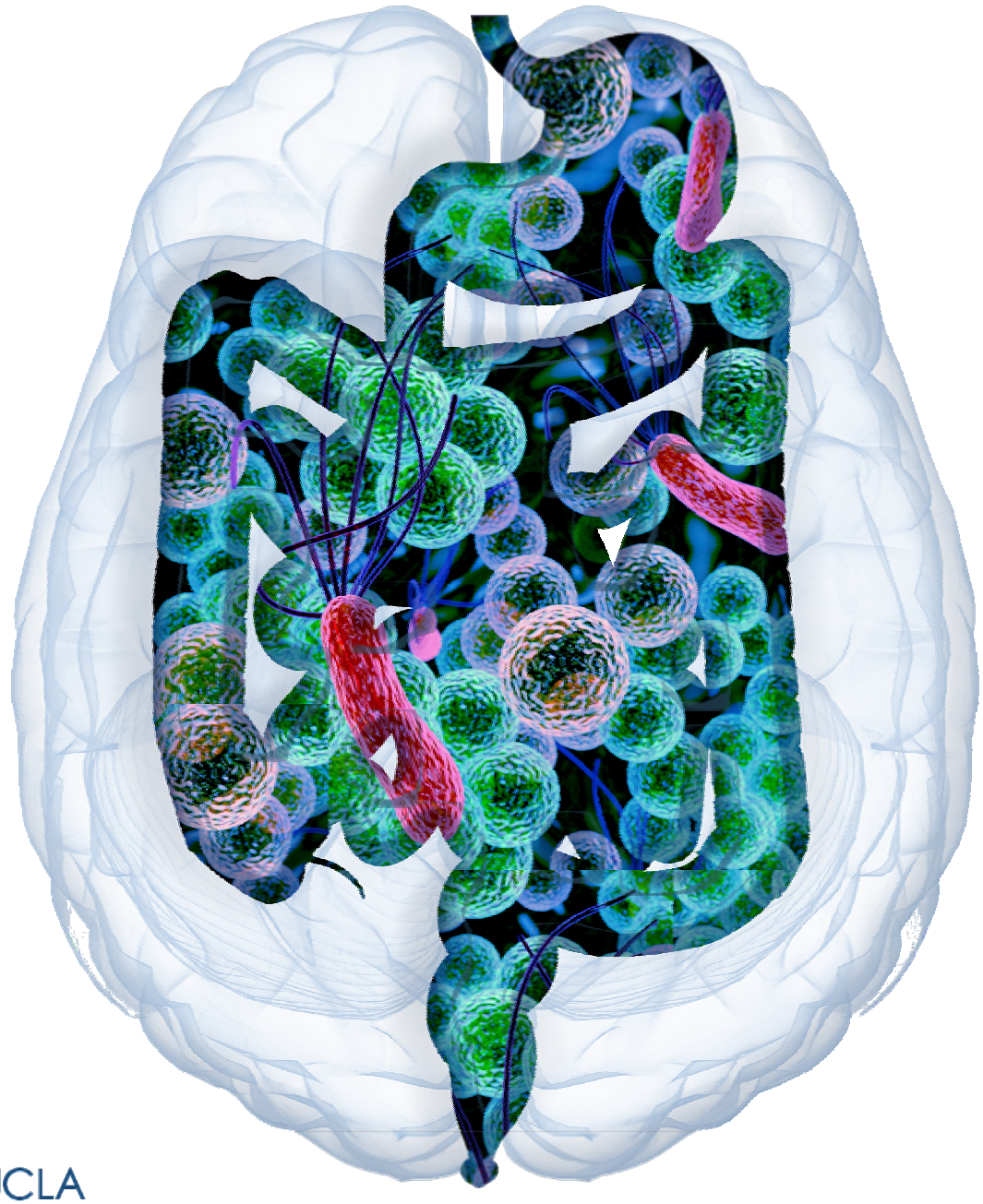
14th Annual CNS
Basic and Translational Science Symposium

BRINGING THE BRAIN BACK INTO MEDICINE: FOCUS ON THE GUT MICROBIOME



UCLA CNS
Gail and Gerald Oppenheimer Family Center for Neurobiology of Stress

February 19, 2016
UCLA California NanoSystems Institute

Acknowledgments

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Jonathan Jacobs, MD, PhD

Claudia Sanmiguel, MD

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Sponsors

We gratefully acknowledge the support for this meeting received from:

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We would also like to acknowledge the ongoing support of the Gail and Gerald Oppenheimer Family Center for Neurobiology of Stress and of this symposium by:

Gerald Oppenheimer Family Foundation

Morris A. Hazan Family Foundation

Samuel P. Mandell Foundation

The UCLA Gail and Gerald Oppenheimer Center for Neurobiology of Stress is supported by **NIH Office of Research on Women's Health (OROWH)** and the **National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)** (**NIH Grant P50 DK064539)** and by individual investigator grants from **NIDDK, NIDA** and **NCCIH**

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14TH ANNUAL CNS BASIC AND TRANSLATIONAL SCIENCE SYMPOSIUM

Bringing the Brain Back into Medicine: Focus on the Gut Microbiome

Gail and Gerald Oppenheimer Family Center for Neurobiology of Stress
Division of Digestive Diseases, Department of Medicine
David Geffen School of Medicine at UCLA

In collaboration with the UCLA California NanoSystems Institute
and Co-Sponsored by the UCLA Microbiome Center

With the generous support from UCLA Brain Research Institute, UCLA Division of Digestive Diseases, VA Greater Los Angeles Healthcare System/Brentwood Biomedical Research Institute, CURE Foundation, Gail and Gerald Oppenheimer Family Foundation and Morris A. Hazan Family Foundation

Friday, February 19, 2016

CALIFORNIA NANOSYSTEMS INSTITUTE AUDITORIUM

Symposium Chairs: **Claudia Sanmiguel, MD** (Director, Ingestive Behavior and Obesity Program – Oppenheimer Center for Neurobiology of Stress, Division of Digestive diseases, David Geffen School of Medicine at UCLA) and **Jonathan Jacobs, MD, PhD** (Clinical Instructor, Division of Digestive Diseases, David Geffen School of Medicine at UCLA)

8:00 am – 8:30 am **CONTINENTAL BREAKFAST**

8:30 am – 8:45 am **INTRODUCTION**

Eric Esrailian, MD, MPH
Lincy Foundation Chair, Clinical Gastroenterology; Co-Chief, Division of Digestive Diseases, David Geffen School of Medicine at UCLA

Stephen Smale, PhD
Distinguished Professor, Microbiology, Immunology and Molecular Genetics; Vice Dean for Research, David Geffen School of Medicine at UCLA

Griffin Rodgers, MD, MACP
Director, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

SESSION I **Session Chair: Jonathan Braun, MD, PhD** (Chair and Professor, Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA)

8:45 am – 9:45 am **STATE OF THE ART LECTURES, PART I**
(Each 25 minutes + 5 minutes discussion)

8:45 am – 9:15 am *Digitizing the Chemistry of Microbes and People through Molecular 3D Cartography*
Pieter C. Dorrestein, PhD
Director, Collaborative Mass Spectrometry Innovation Center; Co-Director, Institute for Metabolomics Medicine, Skaggs School of Pharmacy & Pharmaceutical Sciences, University of California, San Diego

9:15 am – 9:45 am *Defining a Pre-Disease Microbial Risk State for Inflammatory Bowel Disease*
Jonathan Jacobs, MD, PhD
Clinical Instructor, Division of Digestive Diseases, David Geffen School of Medicine at UCLA

9:45 am – 10:15 am *Linking the Brain and the Gut Microbiome in Humans*
Jennifer Labus, PhD
Director, Neuroimaging and Bioinformatics Core – Oppenheimer Center for Neurobiology of Stress, Division of Digestive diseases, David Geffen School of Medicine at UCLA

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Emeran Mayer, MD, PhD (Director, Oppenheimer Center for Neurobiology of Stress; Co-Director, CURE: Digestive Diseases Research Center; Division of Digestive Diseases, David Geffen School of Medicine at UCLA);
Jeffrey Miller, PhD (Director, California NanoSystems Institute, Fred Kavli Chair, NanoSystems Sciences, UCLA);
Jonathan Braun, MD, PhD (Chair and Professor, Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA)

10:30 am – 10:45 am **COFFEE BREAK**

10:45 am – 11:00 am **STATE OF THE CENTER**
Emeran Mayer, MD, PhD
Director, Oppenheimer Center for Neurobiology of Stress; Co-Director, CURE: Digestive Diseases Research Center; Division of Digestive Diseases, David Geffen School of Medicine at UCLA

SESSION II **Session Chairs: Yvette Taché, PhD** (Director, CURE: Animal Models Core; Professor, Division of Digestive Diseases, David Geffen School of Medicine at UCLA) and **Enrique Rozengurt, DVM, PhD** (Director, CURE: Digestive Diseases Research Center; Chief of Research, Division of Digestive Diseases, David Geffen School of Medicine at UCLA)

11:00 am – 12:15 pm **RESEARCH PROGRAMS OF THE CENTER**
(Program highlights and future direction: Each 10 minutes + 5 minutes Q&A)

11:00 am – 11:15 am **SCOR CENTER FOR NEUROVISCERAL SCIENCES AND WOMEN'S HEALTH**
Emeran Mayer, MD, PhD; Lin Chang, MD

11:15 am – 11:30 am **MULTIDISCIPLINARY APPROACHES TO PELVIC PAIN CONSORTIUM (MAPP)**
Larissa Rodriguez, MD

11:30 am – 11:45 am **INGESTIVE BEHAVIOR AND OBESITY PROGRAM**
Claudia Sanmiguel, MD

11:45 am –12:00 pm **PAIN RESEARCH PROGRAM**
Bruce Naliboff, PhD

12:00 pm – 12:15 pm **MIND BODY RESEARCH PROGRAM**
Kirsten Tillisch, MD

12:15 pm – 12:30 pm The UCLA Mind Well Program
Robert M. Bilder, PhD
Director, Mind Well Program, UCLA Healthy Campus Initiative; Michael E. Tennenbaum Family Professor of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Professor of Psychology, UCLA

12:30 pm – 2:00 pm **LUNCH AND POSTER SESSION**

SESSION III **Session Chairs: Charalabos Pothoulakis, MD** (Eli and Edythe Broad Chair, Department of Medicine; Director of Research, UCLA Center for Inflammatory Bowel Diseases, Division of Digestive Diseases, David Geffen School of Medicine at UCLA) and **Jennifer Labus, PhD** (Director, Neuroimaging and Bioinformatics Core – Oppenheimer Center for Neurobiology of Stress, Division of Digestive diseases, David Geffen School of Medicine at UCLA)

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Functional Impact and Neurologic Signature of Centralized Pain
Jason Kutch, PhD
Assistant Professor, Division of Biokinesiology and Physical Therapy, University of Southern California

Unique Microstructural Changes in the Brain Associated with UCPPS Revealed by Diffusion Tensor Imaging and Super-Resolution Tract Density Imaging
Benjamin Ellingson, PhD
Associate Professor of Radiology, David Geffen School of Medicine at UCLA

Integrative Analysis of Colonic Mucosal Microbiome and miRNA Meta-Data in Irritable Bowel Syndrome Patients Compared to Healthy Controls
Swapna Joshi, PhD
Assistant Project Scientist, Oppenheimer Center for Neurobiology of Stress, Division of Digestive Diseases, David Geffen School of Medicine at UCLA

A High Protein Diet Reduces Body Fat Mass and Alters the Gut Microbiome, with Expansion of Akkermansia, in a Rat Model of Diet-Induced Obesity
Lixin Wang, MD, PhD
Associate Researcher, Division of Digestive Diseases, Department of Medicine, David Geffen School of Medicine at UCLA

Sex Differences in the Functional Connectivity of Insular Cortex during Colorectal Distension
Zhuo Wang, PhD
Assistant Professor of Research, Department of Psychiatry and Behavioral Sciences, Keck School of Medicine, University of Southern California

Limited Nesting Stress Alters Maternal Behavior and In Vivo Intestinal Permeability and Is Associated with Loss of Fiber-Digesting and Butyrate-Producing Bacteria in the Intestinal Microbiome at Weaning in Wistar Rat
Nabila Moussaoui, PhD
Post-Doctoral Fellow, Division of Digestive Diseases, David Geffen School of Medicine at UCLA

The Effect of Bariatric Surgery in the Brain-Gut Axis: Post-Operative Changes in Amino Acid Profiles Are Linked to Modifications in the Brain's Reward System Function and Structure and in Eating Behaviors
Claudia Sanmiguel, MD
Director, Ingestive Behavior and Obesity Program – Oppenheimer Center for Neurobiology of Stress, Division of Digestive diseases, David Geffen School of Medicine at UCLA

SESSION IV **Session Chairs: Andrea Rapkin, MD** (Director, Chronic Pelvic Pain and Vulvar Pain Program, Department of Department of Obstetrics and Gynecology, UCLA) and **Kirsten Tillisch, MD** (Director, Mind Body Research Program – Oppenheimer Center for Neurobiology of Stress, Division of Digestive diseases, David Geffen School of Medicine at UCLA)

3:30 pm – 4:30 pm **STATE OF THE ART LECTURES, PART II**
(Each 25 minutes + 5 minutes discussion)

3:30 pm – 4:00 pm *Microbial Modulation of Host Neurochemicals*
Elaine Hsiao, PhD
Assistant Professor, Department of Integrative Biology and Physiology, De Logi Chair in Biological Sciences, Division of Digestive Diseases, Department of Medicine, David Geffen School of Medicine at UCLA

4:00 pm – 4:30 pm *Transmission of Stress Signals: Neurodevelopmental Programming through the Microbiome*
Tracy Bale, PhD
Professor of Neuroscience, Perelman School of Medicine at University of Pennsylvania

4:30 pm – 4:40 pm **POSTER AWARD**
Joseph Pisegna, MD
Chief, Division of Gastroenterology and Hepatology, VA Greater Los Angeles Healthcare System; Professor of Medicine, Division of Digestive Diseases, David Geffen School of Medicine at UCLA

4:40 pm – 5:00 pm **CLOSING COMMENTS**
Lin Chang, MD

5:00 pm **END OF SYMPOSIUM**

Summaries of Presentations

Symposium Chairs: Jonathan Jacobs, MD, PhD (Financial Disclosure: None)
Claudia Sanmiguel, MD (Financial Disclosure: None)

SESSION I: STATE OF THE ART LECTURES, PART I

Chairs: Jonathan Braun, MD, PhD (Financial Disclosure: None)

Digitizing the Chemistry of Microbes and People through Molecular 3D Cartography

Pieter C. Dorrestein, PhD
Director, Collaborative Mass Spectrometry Innovation Center; Co-Director, Institute for Metabolomics Medicine, Skaggs School of Pharmacy & Pharmaceutical Sciences, University of California, San Diego
Financial Disclosure: None

It is becoming clear that the microbiome plays critical roles in human health but to understand the roles microbes play and their relationship to the chemistries in our bodies is still poorly understood. UCSD has recently launched the microbiome center. The Collaborative Mass Spectrometry Innovation Center plays a key and integrated role in this center to begin unraveling the chemistry of the microbiome. In this presentation we will highlight the latest mass spectrometry based tools, including our crowd source molecular annotation platform, to study the chemistry and microbiome of the skin, gut and lungs of mice and people in relationship to clinical information.

Defining a Pre-Disease Microbial Risk State for Inflammatory Bowel Disease

Jonathan Jacobs, MD, PhD
Clinical Instructor, Division of Digestive Diseases, David Geffen School of Medicine at UCLA
Financial Disclosure: None

The intestinal microbiome is widely believed to have a pathogenic role in inflammatory bowel disease (IBD). However, it remains unclear which differentially abundant microbes in IBD patients are instigators of disease rather than bystanders responding to the altered environment of the inflamed intestine. If IBD develops as a consequence of a host response to a pro-inflammatory microbiome and its associated microbial products, as has been reported in animal models, IBD patients would be predicted to harbor dysbiosis prior to the development of disease. We investigated this hypothesis in a family based study characterizing the fecal microbiome and metabolome of pediatric IBD patients in clinical remission and their first degree relatives, a population at high risk for developing IBD. We found that subjects grouped into one of two states based on fecal microbial and metabolomics profiles, which could be bioinformatically defined as enterotypes and metabotypes (multidimensional clusters characterized by differences in the abundance of signature taxa or metabolites). These enterotypes and metabotypes were highly correlated with one another, and with disease status. The IBD-associated enterotype was characterized by lower microbial diversity and increased Lachnospiraceae and Enterobacteriaceae irrespective of disease status. Healthy relatives with the IBD-associated enterotype had an increased incidence of elevated fecal calprotectin. We propose that these individuals harbor a stable pre-disease microbial/metabolomic risk state for IBD. This “dysbiosis” could arise due to genetic risk factors for IBD that affect immune gardening of the intestinal microbiome and epithelial barrier function. This possibility is being explored in an ongoing human cohort study investigating the association of common IBD-associated genetic polymorphisms with microbial features of IBD in the colonic mucosa of healthy controls. The mechanisms underlying association of specific IBD-associated genes such as RORC with pre-disease dysbiosis are being investigated further using knockout mice.

Linking the Brain and the Gut Microbiome in Humans

Jennifer Labus, PhD
Director, Neuroimaging and Bioinformatics Core – Oppenheimer Center for Neurobiology of Stress, Division of Digestive diseases, David Geffen School of Medicine at UCLA
Financial Disclosure: None

Preclinical data suggests a relationship between gut microbiota and their metabolites with brain signaling systems, as well as emotional, nociceptive, social and feeding behaviors. Bidirectional signaling occurs between the brain and the gut microbiome through multiple neural, immune, and endocrine signaling mechanisms. In this talk, Dr. Labus will review human brain gut microbiome studies that support the concept of a brain gut microbiome axis in human subjects. Dr. Labus will present results from ongoing research at the UCLA Oppenheimer Center of Neurobiology of Stress demonstrating association between chronic abdominal pain, microbial composition, metabolite concentrations, and brain structure. Her talk will highlight some of the most exciting analytical approaches to linking brain imaging with gut microbes. In her talk, she will emphasize the need for future, more mechanistic and longitudinal experimental designs based on hypothesis generating cross-sectional studies.

The UCLA Microbiome Center

Emeran Mayer, MD, PhD
Director, Oppenheimer Center for Neurobiology of Stress; Co-Director, CURE: Digestive Diseases Research Center; Division of Digestive Diseases, David Geffen School of Medicine at UCLA
Financial Disclosure: Dannon and Danone Advisory Board Member

Jeffrey Miller, PhD
Director, California NanoSystems Institute, Fred Kavli Chair, NanoSystems Sciences, UCLA
Financial Disclosure: None

Jonathan Braun, MD, PhD
Chair and Professor, Pathology and Laboratory Medicine, , David Geffen School of Medicine at UCLA
Financial Disclosure: None

The UCLA Microbiome Center (www.microbiome.ucla.edu) brings together UCLA investigators from different UCLA departments, schools and from the California NanoSystems Institute interested in the human microbiome,representing a wide spectrum of expertise, spanning from oral biology, mucosal inflammation, metabolism, skin, to brain gut interactions. The main goal of the Center is to provide a home for microbiome interested investigators which will foster interdisciplinary interactions. In order to accomplish this goal, we plan to develop coordination opportunities for technology use and use of advanced technology platforms (e.g., bioinformatics, 16s analyses, metabolomics), provide a forum for interdisciplinary seminars and lectures, provide opportunities for students, trainees and junior faculty members to develop expertise in the microbiome field, and identify and facilitate action on funding opportunities for large scale multidisciplinary research projects.

SESSION II: RESEARCH PROGRAMS OF THE CENTER

Chairs: Yvette Taché, PhD (Financial Disclosure: None)
Enrique Rozengurt, DVM, PhD (Financial Disclosure: None)

SCOR: CENTER FOR NEUROVISCERAL SCIENCES AND WOMEN'S HEALTH

Emeran Mayer, MD, PhD

Lin Chang, MD
Financial Disclosure: Takeda, Commonwealth Laboratories, QOL Medical, AstraZeneca, Bioamerica, Synergy, Ardelyx

Mission: To advance the science, practice and teaching of brain visceral interactions, focusing on women's health and sex-related differences through interdisciplinary translational approaches, with the ultimate goal of improving the treatment of patients with functional visceral pain disorders, in particular IBS.

Background and Rationale:

- This research program funded by NIDDK and ORWH aims to characterize changes in the way the brain and gut communicate with each other in health and in irritable bowel syndrome (IBS) with a special emphasis on identifying the role of stress and sex related differences in these interactions.
- The UCLA SCOR proposes a unique translational and interdisciplinary approach to achieve this goal, ranging from molecular studies to multimodal brain imaging techniques in humans and rodent models. The long term goal of the proposed studies is to identify subgroups of IBS patients which may respond differentially to different therapies.
- Researchers apply cutting edge technologies to unravel the structure and function of the brain, the immune system, stress response systems and the microorganisms living in our intestine
- The studies will help to more effectively treat many chronic gastrointestinal disorders with pharmacological and non-pharmacological treatments.

MULTIDISCIPLINARY APPROACHES TO PELVIC PAIN CONSORTIUM (MAPP)

Larissa Rodriguez, MD

Financial Disclosure: None

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) established the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network to advance scientific knowledge regarding chronic urologic pelvic pain. MAPP embraces a systemic, or whole-body, approach and integrates collaborative longitudinal studies of sex differences, biomarkers, psychosocial variables and brain imaging in pursuit of better understanding and treatment of these common but complex disorders. In addition MAPP illustrates a highly successful model of large scale, multisite, and multidisciplinary scientific collaboration.

INGESTIVE BEHAVIOR AND OBESITY RESEARCH PROGRAM

Claudia Sanmiguel, MD

Mission: To advance the understanding of the role of the human brain in the regulation of ingestive behaviors in health and in disease including obesity and eating disorders. We place emphasis in the role of bidirectional communications in the gut-brain axis using innovative research tools and a multisystems approach including neuroimaging, metabolomics, and psychophysiological approaches among others.

Background and Rationale:

- The control of food intake is one of the most highly adaptive and regulated biological processes, and the high prevalence of disorders in industrialized societies points towards strong environmental factors
- For both obesity and eating disorders, alterations in bidirectional brain gut interactions have been proposed as plausible disease models
- The complexity of the regulation of the body weight and eating behaviors calls for a multisystems approach to elucidate pathways implicated in health and in disease

PAIN RESEARCH PROGRAM

Bruce Naliboff, PhD

Financial Disclosure: None

Mission: To advance our understanding of brain-body interactions important for the development, maintenance and alleviation of chronic pain. To develop novel brain imaging and behavioral technologies for use in chronic pain research and treatment

Background and Rationale:

- Chronic pain affects an estimated 116 million American adults and costs the nation up to $635 billion each year in medical treatment and lost productivity.
- This program integrates cutting edge brain research using neuroimaging with insights from behavioral sciences to provide new answers for critical questions in chronic pain.

Major Projects:

- Establishment of the CNS Multisite Data Core to promote reliable gathering, archiving and analysis of brain imaging data collected at multiple sites as part of large scale collaborative research networks.
- Development of the first NIH funded repository of brain imaging in chronic pain to identify "brain signatures" (changes in brain structure and function) associated with various chronic pain conditions
- Novel studies to identify brain signatures of resilience

Disease Areas: Irritable Bowel Syndrome, Inflammatory Bowel Diseases, Chronic Pelvic Pain, Vulvodynia, Fibromyalgia

MIND BODY RESEARCH PROGRAM

Kirsten Tillisch, MD
Financial Disclosure: None

Mission: To promote excellence and innovation in Mind-Body medicine at through fostering new research on the neurobiology of health and healing for chronic pain disorders.

Background and Rationale:

- Mind-Body medicine includes a broad range of interventions to improve health and treat disease, including meditation, hypnosis, cognitive behavioral therapy, energy therapies, yoga and tai chi
- Our program applies cutting edge science to Mind Body interventions for health and wellbeing with the goal of understanding their biological mechanisms

Major Projects:

- An NIH funded study to understand the neurobiology of mindfulness on chronic pain symptoms and gut microbiota
- Pilot programs to evaluate the benefits of yoga and tai chi in Veterans
- Study of cranial electrical stimulation therapy on mood and wellbeing

The UCLA Mind Well Program

Robert M. Bilder, PhD
Director, Mind Well Program, UCLA Healthy Campus Initiative; Michael E. Tennenbaum Family Professor of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Professor of Psychology, UCLA
Financial Disclosure: None

The UCLA Healthy Campus Initiative (HCI) is committed to making UCLA a world leader in advancing the well-being of our students, staff and faculty. The HCI comprises a series of program areas or "pods" that work together to achieve these goals. Our Eat Well pod focuses on diet and nutrition. Move Well targets physical activity and exercise. BE Well addresses the built environment to advance health goals. Mind Well aims to promote wellness of mind, brain and spirit, foster creativity, and enhance social connectedness throughout the UCLA community. The Mind Well program has grown from a concept first articulated in 2011 when Semel Institute leaders first presented the idea to the upper campus, and has grown over the last 5 years to include a broad range of programs and activities. The Mindful Awareness Research Center (MARC) has grown dramatically and now offers a range of "drop in" classes, formal courses, and other events and experiences that enable meditation practices to flourish. Mind Well has convened summer session classes including Personal Brain Management, Integrative East West Medicine, and Mindfulness Practice and Theory. We launched the Mindful Music program to engage the campus in "pop up" live music performances. Our Sleep Campaign focused on offering nap spaces and sleep hygiene education to our campus. A Brain Fitness Center has helped people on campus learn about the realities of "brain training" and personal EEG devices. Student Research Projects have helped students advance their own understanding of research on mind, body and their intersection. We helped reinstitute the "Life Skills" classes that have been a favorite of students and a boon to well-being for years to come. The Mind Well program is now investigating new programs and curricula to establish UCLA as a leader in the teaching of "eudaimonia"; a term Aristotle used to describe the sustained satisfaction that comes from living

a life full of purpose and meaning. By facilitating, coordinating, and promoting the development of these programs, we hope to have a lasting impact on our UCLA community and beyond.

SESSION III: DATA BLITZ – RESEARCH HIGHLIGHTS

Chairs: Charalabos Pothoulakis, MD (Financial Disclosure: None)
Jennifer Labus, PhD

Unique Microstructural Changes in the Brain Associated with UCPPS Revealed by Diffusion Tensor Imaging and Super-Resolution Tract Density Imaging

Benjamin Ellingson, PhD
Associate Professor of Radiology, David Geffen School of Medicine at UCLA
Financial Disclosure: None

Studies have suggested chronic pain syndromes are associated with neural reorganization in specific regions associated with perception, processing, and integration of pain. Urological chronic pelvic pain syndrome (UCPPS) represents a collection of pain syndromes characterized by pelvic pain, namely Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) and Interstitial Cystitis/Painful Bladder Syndrome (IC/PBS), that are both poorly understood in their pathophysiology, and treated ineffectively. We hypothesized patients with UCPPS may have microstructural differences in the brain compared with healthy control subjects (HCs), as well as patients with irritable bowel syndrome (IBS), a common gastrointestinal pain disorder. In the current study we performed population-based voxel-wise DTI and super-resolution track density imaging (TDI) in a large, two-center sample of phenotyped patients from the multicenter cohort with UCPPS (N=45), IBS (N=39), and HCs (N=56) as part of the MAPP Research Network. Compared with HCs, UCPPS patients had lower fractional anisotropy (FA), lower generalized anisotropy (GA), lower track density, and higher mean diffusivity (MD) in brain regions commonly associated with perception and integration of pain information. Results also showed significant differences in specific anatomical regions in UCPPS patients when compared with IBS patients, consistent with microstructural alterations specific to UCPPS. While IBS patients showed clear sex related differences in FA, MD, GA, and track density consistent with previous reports, few such differences were observed in UCPPS patients. Heat maps illustrating the correlation between specific regions of interest and various pain and urinary symptom scores showed clustering of significant associations along the cortico-basal ganglia-thalamic-cortical loop associated with pain integration, modulation, and perception. Together, results suggest patients with UCPPS have extensive microstructural differences within the brain, many specific to syndrome UCPPS versus IBS, that appear to be localized to regions associated with perception and integration of sensory information and pain modulation, and seem to be a consequence of longstanding pain.

Functional Impact and Neurologic Signature of Centralized Pain

Jason Kutch, PhD
Assistant Professor, Division of Biokinesiology and Physical Therapy, University of Southern California
Financial Disclosure: None

Background: Chronic pain impact is often measured with an overall severity score which ignores the spatial distribution of pain across the body. Widespread body pain has been hypothesized to be an important marker of a pain centralization process that decouples pain perception from nociceptive input. Here we measure the reduction in physical function associated with widespread body pain, as well as identify and validate its neurologic signature across chronic pain disorders in the human brain. **Methods:** Data were analyzed from 1079 participants in the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network study. The primary cohort of participants had a clinical diagnosis of Urological Chronic Pelvic Pain Syndrome (UCPPS). An additional cohort of pain-free controls and patients with a clinical diagnosis of fibromyalgia, the prototypical centralized pain state, were used for validation. All participants completed questionnaires to capture self-reports of physical function and painful body locations. Brain structural and functional magnetic resonance imaging (MRI) data were also acquired in 318 of the participants. MRI data were analyzed to identify and validate a neurologic signature of

centralized pain. **Results:** UCPPS patients exhibit a spectrum of pain widespreadness, ranging from highly localized pelvic pain to pain widely distributed across the body. Widespread pain impacts daily function over and above the impact of overall pain severity. UCPPS patients with widespread pain display markers of altered brain structure and functional connectivity focused in the sensorimotor and insular cortices, suggesting a central nervous system pathology. This neurologic signature of widespread pain is valid beyond UCPPS, as these markers show similarities in patients with fibromyalgia. **Conclusion:** Widespread pain degrades physical function in patients with chronic pain. Widespread pain also has an objective neurologic signature in the brain that generalizes across clinical diagnoses. We speculate that identifying which patients display this signature could guide existing and de novo treatment strategies.

Integrative Analysis of Colonic Mucosal Microbiome and miRNA Meta-Data in Irritable Bowel Syndrome Patients Compared to Healthy Controls

Swapna Joshi, PhD
Assistant Project Scientist, Oppenheimer Center for Neurobiology of Stress, Division of Digestive Diseases, David Geffen School of Medicine at UCLA
Financial Disclosure: None

The role of human digestive-tract associated microbes, known as gut microbiome in both health and disease has been the subject of extensive research. Altered gut microbiota have been linked with gastrointestinal conditions such as irritable bowel syndrome (IBS). IBS is a prevalent gastrointestinal disorder characterized by abdominal pain or discomfort and altered bowel habits. The role of host miRNAs, which are small non-coding RNAs that regulate gene expression, in selectively shaping gut microbiota of IBS patients is largely unclear. A recent study by Liu et al. identified targeting of microbiota by fecal miRNA as a defense mechanism that regulates gut microbiota. Aims of the present study were to identify dysbiosis (changes in composition of microbiota) as well as differential expression of miRNA associated with IBS, and to bioinformatically assess the relationship between gut microbiome and miRNA. We assessed alpha and beta diversity of colonic mucosal microbiome via 16srRNA sequencing, in IBS patients and healthy controls. Further, we attempted to correlate the differential expression of miRNAs with the richness of most abundant bacteria.

A High Protein Diet Reduces Body Fat Mass and Alters the Gut Microbiome, with Expansion of Akkermansia, in a Rat Model of Diet-Induced Obesity

Lixin Wang, MD, PhD
Researcher, Division of Digestive Diseases, Department of Medicine, David Geffen School of Medicine at UCLA
Financial Disclosure: None

A high protein diet (HPD) can reduce diet-induced obesity in animal models and in humans. It is known that the compositions of the gut microbiome are altered in obese rodents and humans, and by high fat and high sugar diet – Western diet (WD). However, it is unknown whether HPD affects the microbiome altered by WD. We found that rats on a WD for at 12 weeks had 70% higher body fat mass than rats on normal diet. Switching to a HPD reduced fat by 29% after 6 weeks compared to rats remaining on WD. The WD and HPD groups had comparable microbial diversity, which was increased relative to rats on normal diet as measured by the Shannon index, Chao1 richness, and phylogenetic diversity. Weighted UniFrac analysis demonstrated a shift in the intestinal microbiome composition of the HPD group compared to the WD group ($p<10\text{-}5$). The HPD group had increased abundance of 114 OTUs at a significance threshold of $q<0.05$, including Akkermansia mucinophila, Fusobacterium, segmented filamentous bacteria, Ruminococcus (21 OTUs), and Bacteroides (14). Only Akkermansia mucinophila ($q=0.008$) and an unclassified Clostridiales ($q=0.04$) had a statistically significant inverse correlation with fat mass after adjustment for diet. There was decreased abundance of 188 OTUs, including Lactobacillus (14), Clostridium (6), Bifidobacterium (4), Roseburia (2), and Turicibacter (2). Of these, an unclassified RF39 ($q=0.0001$) and a Phascolarctobacterium ($q=0.02$) were correlated with fat mass. Our data show that reduction of fat mass by a high protein diet is associated with extensive shifts in the intestinal microbiome. To further investigate whether the expanded bacteria correlated to body fat mass are beneficial for weight loss will help with developing therapy for obesity.

Sex Differences in the Functional Connectivity of Insular Cortex during Colorectal Distension

Zhuo Wang, PhD
Assistant Professor of Research, Department of Psychiatry & Behavioral Sciences, Keck School of Medicine, University of Southern California
Financial Disclosure: None

Background: The insular cortex plays a critical role in visceral pain processing and shows sex differences in functional activation during noxious visceral stimulation. Much less is known at the circuit level regarding functional interactions both within the insula and between the insula and other parts of the brain. **Aim:** To characterize sex differences in insular functional connectivity (FC) in rats during noxious colorectal distension (CRD). **Methods:** Functional connectivity analysis was applied to a published data set consisting of 4 groups: male/control, male/distended, female/control, and female/distended. Cerebral blood flow mapping was performed using 14C-iodoantipyrine autoradiography in awake, nonrestrained rats. Forty regions of interest (ROIs) were defined anatomically to represent the granular, dysgranular, and agranular insular cortex along the anterior-posterior axis. A 40x40 inter-regional correlation matrix was calculated for each group to characterize intra-insular FC, which was further analyzed with graph theoretical tools. Representative insular ROIs were chosen for seed correlation analysis to examine sex differences in their global FC patterns. **Results:** Both control females and males showed strong intra-insular FC with females showing higher density (fraction of connections to possible connections) at 54% compared to males at 32%. Clear functional segregation was seen along the anterior-posterior axis. Denser FC was observed anteriorly in females but posteriorly in males. During CRD, intra-insular FC density decreased greatly to 25% in females, and modestly to 26% in males. A loss of long-range connections was apparent. New functional organization was characterized in both males and females by a functionally connected mid-insular cluster and primarily short-range FC along the anterior-posterior axis of the insula. Seed correlation analysis revealed complex sex- and CRD-related differences in insular FC with other brain areas. In particular, during CRD, sex differences were noted in FC of the anterior agranular insular cortex with the medial prefrontal cortex and with the periaqueductal gray, suggesting sex differences in the affective and modulatory aspects of visceral pain processing. **Conclusions:** Functional connectivity analysis revealed important sex differences in the functional organization of the insular cortex and in its interaction with other areas in the pain circuit. These findings bring new insights into understanding at the circuit-level sex differences in visceral pain processing.

Limited Nesting Stress Alters Maternal Behavior and In Vivo Intestinal Permeability and Is Associated with Loss of Fiber-Digesting and Butyrate-Producing Bacteria in the Intestinal Microbiome at Weaning in Wistar Rat

Nabila Moussaoui, PhD
Post-Doctoral Fellow, Division of Digestive Diseases, David Geffen School of Medicine at UCLA
Financial Disclosure: None

Background: Early life adverse events predispose to stress related intestinal disorders such as irritable bowel syndrome (IBS). **Aims:** To investigate alterations of maternal behaviors induced by LNS from postnatal day (PND) 2 to 9 in rats and the impact on stress hormone, glycemia and *in vivo* IP on pups and fecal microbiota. **Methods:** Dam Wistar rats were housed under control (direct contact bedding n=9) or LNS conditions (wire bottom floor + ½ paper towel, n=9). Maternal behavior was assessed once daily between 9:00-10:00 am. At PND10 and PND21, blood was collected and IP was assessed 4-h after fluorescein isothiocyanate–dextran 4kDa (FD4) oral gavage. Feces of PND21 rats underwent DNA extraction and amplification of the V4 region of the 16S rRNA gene. Paired end 2x150bp sequencing was performed using an Illumina MiSeq. Alpha and beta diversity analysis was performed at 97% operational taxonomic units (OTUs) and multivariate analysis with DESeq2. **Results:** Dams with LNS spent similar time licking and grooming pups and eating/drinking while spending more time building a nest (118%), self-grooming (69%), and bringing pups to the nest (167%) compared to CTL. Frequency of pups outside of nest was 3.7 times higher in LNS than CTL. At PND10, LNS male and female pups had reduced body weight (4-5%), adrenal weights/100g BW (17-18%), plasma corticosterone levels (64-62%) and blood glucose (11%-12%) vs. same sex CTL. IP was increased only in male LNS pups by 2.7-fold. At weaning, the LNS group maintained the body weight reduction and hypercorticosteronemia (males 67 %; females147% vs. CTL) and had increased IP only in females (1.7-fold vs CTL, $P<0.01$). CTL showed no sex difference either PND10 or 21. At weaning, the LNS group showed increased fecal microbial diversity using three metrics: Chao1 ($p=0.001$), phylogenetic diversity ($p=0.002$), and Shannon index ($p=0.001$). Unweighted UniFrac analysis indicates that LNS rats had a distinct fecal microbiome composition compared to controls ($p=0.0008$) with

decreased abundance of 24 genera (q<0.05): fiber-degrading microbes: *Oscillospira* (47 OTUs), *Ruminococcus* (17), and *Lachnospira* (3); butyrate-producing microbes: *Roseburia* (27), *Coprococcus* (10), and *Eubacterium dolichum*; and mucus-resident bacteria *Akkermansia mucinophila* and *Mucispirillum schaedleri*. There was increased abundance of 20 genera, of which 8 were Gram positive cocc. Additional enriched genera included *Clostridium* (4), *Corynebacterium* (4), *Desulfovibrio* (1), *Granulicatella* (2), *Rothia* (1), and *Proteus* (1). No genera and only 2 OTUs were significantly sex associated. **Conclusions:** LNS delayed the HPA maturation of PND10 followed by elevated corticosteronemia at weaning. The alterations of fecal microbiota with expansion of several genera of Gram positive cocci along with sex dependent elevation of intestinal permeability induced by LNS at PND10 and weaning may contribute to the sex-related susceptibility to IBS.

The Effect of Bariatric Surgery in the Brain-Gut Axis: Post-Operative Changes in Amino Acid Profiles Are Linked to Modifications in the Brain's Reward System Function and Structure and in Eating Behaviors

Claudia Sanmiguel, MD

Bariatric surgery is the most effective treatment for sustained weight loss, however we a poor understanding on how this sustained weight loss is achieved. Recent studies have shown that gastric bypass and sleeve gastrectomy result in modifications in eating behaviors and food preferences but the mechanisms underlying those effects are not known. We hypothesized that changes in the brain regulation of eating behaviors after surgery play a role in weight loss and that those changes are associated with modifications in the metabolite environment and in gut microbiome/brain axis after surgery. In this study we assess the role of postoperative changes in amino acids profiles with emphasis in aromatic amino acids, on brain function/structure at the reward system regions and on hedonic eating behaviors. We also explore the role of gut microbiome modifications in changes in this metabolite profiles.

SESSION IV: STATE OF THE ART LECTURES, PART II

Chairs: Andrea Rapkin, MD (Financial Disclosure: None)
Kirsten Tillisch, MD

Microbial Modulation of Host Neurochemicals

Elaine Hsiao, PhD
Assistant Professor, Department of Integrative Biology and Physiology, De Logi Chair in Biological Sciences, Division of Digestive Diseases, Department of Medicine, David Geffen School of Medicine at UCLA
Financial Disclosure: None

There is growing evidence that the microbiota fundamentally regulates the development and function of the nervous system, but the mechanisms underlying indigenous microbe-nervous system interactions are largely unknown. We explore fundamental interactions between the indigenous microbiota and mammalian host that regulate the bioavailability of neuroactive molecules, including neurotransmitters and neuropeptides. In particular, we reveal that a striking ~60% of peripheral serotonin (5-hydroxytryptamine, 5-HT) is regulated by the microbiota. We have identified a limited microbial consortium that sufficiently and reversibly modulates host serotonin biosynthesis in specific cell subtypes of the gastrointestinal tract, and that corrects enteric and hemostatic abnormalities related to serotonin deficiency in germ-free and genetically-altered mice. We further identify particular microbial metabolites that confer this serotonergic effect of gut microbes, representing the first account of the molecular mechanisms by which a limited bacterial consortium from the mouse or healthy human microbiota modulates host serotonin levels and serotonin-related disease phenotypes in mice.

Transmission of Stress Signals: Neurodevelopmental Programming through the Microbiome

Tracy Bale, PhD
Professor of Neuroscience, Perelman School of Medicine at University of Pennsylvania
Financial Disclosure: None

Stress pathway dysregulation is the most pervasive symptom in neuropsychiatric disease, yet we understand little as to the developmental programming and maturation of this system and the sensitive periods during which perturbations may be disruptive. Stress during pregnancy is associated with an increased risk of neurodevelopmental disorders. The mechanisms through which fetal antecedents contribute to disease development involve complex interactions between the maternal and fetal environments where a key factor is the transfer of the maternal vaginal microbiota to the neonate during vaginal birth. As the neonate's gut is initially populated by this inoculant, changes produced by maternal stress to the diversity of the vaginal environment and to neonate gut development can dramatically alter this initial interaction following birth. Neonatal gut ecology has critical influences on education of the host immunity, development of the enteric nervous system, and on brain development and behavior.

Using targeted approaches in our mouse model of early prenatal stress (EPS), we have examined the maternal vaginal and offspring gut microbiome for changes in diversity and composition as well as specific levels of Lactobacillus, the most common species between vaginal and gut microbiota. In addition, we have used metabolomics analyses to examine the link between neonate gut microbiota and nutrient absorption by measuring serum extractions for both free fatty acids and water-soluble metabolites. At these same neonatal time points, we have also examined the hypothalamus by transcriptomics for programmatic changes related to an altered gut microbiome during development.

To demonstrate causality, we have utilized c-section delivery and oral gavage of maternal vaginal samples in this model. We found that EPS affects both the maternal vaginal and offspring gut microbiome diversity and content in sex-specific manner, highlighting the importance of both the presentation of microbiota and the gut receptivity. Neonate serum samples again showed intriguing sex differences resulting from prenatal stress that supports functional differences in gut microbial diversity. This altered microbiota composition in the neonate gut corresponded with changes in metabolite profiles involved in energy balance, and with region- and sex-specific disruptions of amino acid profiles in the developing hypothalamus. We identified male EPS-specific hypothalamic gene expression patterns with monotonic relationships to neonate gut levels of Lactobacillus, a genus of bacteria known to have neuromodulating properties. C-section studies further supported the critical importance of this initial vaginal microbial inoculant in promoting the metabolic and neurodevelopmental changes following EPS, where the neonate male gut was the determining factor in the final microbiome composition. These studies demonstrate the important link between the maternal stress experience during pregnancy and the vaginal microbiome in populating the offspring gut at birth, and the profound effect this microbial population has on early brain development.

About the Speakers

Tracy Bale, PhD
Professor of Neuroscience, Perelman School of Medicine at University of Pennsylvania

Tracy L. Bale is Professor of Neuroscience in the School of Veterinary Medicine and in the Department of Psychiatry of the Perelman School of Medicine. Her research focuses on understanding the role of stress dysregulation in neurodevelopmental and neuropsychiatric diseases, and the sex differences that underlie disease vulnerability using mice as the model organism. Mechanistic examination includes studies on the contributions of the placenta, germ cells and the microbiome in epigenetic programming of the brain. Dr. Bale is the Co-Director of the Penn Center for the Study of Sex and Gender in Behavioral Health, which is funded by a NIMH and ORWH SCOR P50 grant, and is the Director of Research for the BIRCWH Faculty Scholars. She serves on many internal and external advisory Committees, Panels, and Boards and is currently a Reviewing Editor at the Journal of Neuroscience and serves as Chair of the NNRS CSR study section. She has been the recipient of several awards for her research in this area including the career development award for early career achievement and promise by the Society for Neuroscience, the Richard E. Weitzman Memorial award as exceptionally promising young investigator award by the Endocrine Society, and Medtronic Award from the Society for Women's Health Research for outstanding research that has led to the improvement of women's health.

Robert M. Bilder, PhD
Director, Mind Well Program, UCLA Healthy Campus Initiative; Michael E. Tennenbaum Family Professor of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Professor of Psychology, UCLA

Dr. Bilder received a bachelor's degree from Columbia College of Columbia University in Biology and Psychology (1978), and a Ph.D. in Psychology from City College, City University of New York, where he specialized in human neuropsychology (1984). He did his Internship in the Division of Neuropsychology, New York State Neurological Institute, Columbia-Presbyterian Medical Center (1982). Dr. Bilder has long been dedicated to transdisciplinary research on brain-behavior relations along with the teaching and clinical practice of neuropsychology. After completing his doctoral work in New York, he studied schizophrenia and related disorders using neuropsychological, neuroimaging, and neurophysiological methods, and developed clinical training programs in neuropsychology. After moving to UCLA in 2002, he assumed leadership of the Division of Medical Psychology – Neuropsychology, which governs psychological services and training in the UCLA Health System. He directed the Consortium for Neuropsychiatric Phenomics, supported by the NIH Roadmap Initiative to investigate cognitive phenotypes across levels of analysis from genome to syndrome, and now leads a project under the aegis of the NIMH Research Domains Criteria (RDoC) initiative to examine working memory across diagnostic syndromes. Dr. Bilder additionally heads the Tennenbaum Center for the Biology of Creativity, studying creative cognition and exceptional abilities that may be keys to achievement in diverse artistic, scientific, and other creative domains. He also directs the UCLA Healthy Campus Initiative's Mind Well program, which aims to help promote well-being and creative achievement throughout the UCLA campus community and beyond.

Jonathan Braun, MD, PhD
Chair and Professor, Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA

Dr. Jonathan Braun is Professor and Chair of Pathology and Laboratory Medicine, and Professor of Molecular and Medical Pharmacology at the David Geffen School of Medicine, and Chair of Pathology and Laboratory Medicine. A distinguished pathologist and mucosal immunologist , his 30 year career has been devoted to mucosal host-microbial interaction and the immune cell biology of chronic inflammatory disease (IBD and HIV) and lymphoma pathogenesis. With a long-standing commitment to inflammatory bowel disease, in recent years he has focused on the relationship of the intestinal microbiome and function to human genetic disease variation in IBD disease pathogenesis, penetrance, and phenotype. He has innovated in the detection and bioinformatics analysis of microbiome, metabolites, and peptides, through participation in the NIDDK IBD Genetics Consortium and NIH HMP2 projects, and as PI of the CCFA Microbiome Initiative.

Lin Chang, MD
Director, Functional GI Disorders Program - Oppenheimer Center for Neurobiology of Stress, Division of Digestive Diseases, David Geffen School of Medicine at UCLA

Lin Chang, MD, is a Professor of Medicine in the Department of Medicine, Division of Digestive Diseases, at the David Geffen School of Medicine at UCLA. She serves as the Co-Director of the Oppenheimer Family Center for Neurobiology of Stress at the David Geffen School of Medicine at UCLA. She serves as Program Director of the UCLA Gastroenterology Fellowship Program and Director of the Digestive Health and Nutrition Clinic at UCLA. Dr. Chang's clinical expertise is in functional gastrointestinal disorders. She is a funded NIH-investigator studying the central and peripheral mechanisms underlying IBS. Specifically, her research is focused on the pathophysiology of IBS related to stress including early adverse life events, sex differences, genetic and epigenetic factors, and the treatment of IBS.

Dr. Chang is the recipient of the Janssen Award in Gastroenterology for Basic or Clinical Research and the AGA Distinguished Clinician Award, Dr. Chang has authored 90 original research articles, 50 review articles, and 20 book chapters on her specialty interests. She is Past-President of the American Neurogastroenterology and Motility Society (ANMS), and is also a member of the Rome Foundation Board of Directors, Rome IV Editorial Board and the Functional Bowel Disorders Committee. Dr. Chang is a fellow of the American Gastroenterological Association and American College of Gastroenterology, and a member of the Society for Neuroscience, and is an Associate Editor of the American Journal of Gastroenterology. She is a member of the FDA GI Advisory Panel. She frequently speaks at national and international meetings.

Pieter C. Dorrestein, PhD
Director, Collaborative Mass Spectrometry Innovation Center; Co-Director, Institute for Metabolomics Medicine, Skaggs School of Pharmacy & Pharmaceutical Sciences, University of California, San Diego

Dr. Dorrestein is Professor at the University of California - San Diego. He is the Director of the Collaborative Mass Spectrometry Innovation Center and a Co-Director, Institute for Metabolomics Medicine in the Skaggs School of Pharmacy & Pharmaceutical Sciences, and Department of Pharmacology. Since his arrival to UCSD in 2006, Dr. Dorrestein has been pioneering the development of mass spectrometry methods to study the chemical ecological crosstalk between population of microorganisms, including host interactions for agricultural, diagnostic and therapeutic applications.

Benjamin Ellingson, PhD
Associate Professor of Radiology, David Geffen School of Medicine at UCLA

Dr. Ellingson is the Director of the UCLA Brain Tumor Imaging Laboratory (BTIL), Co-Director of the Center for Computer Vision and Imaging Biomarkers, and a member of the Center for Neurobiology of Stress (CNS). Dr. Ellingson's research involves the development, testing, and implementation of advanced MRI and PET imaging biomarkers for the characterization of biology and therapeutic response assessment in syndromes related to neurooncology, neuropathic pain, neurotrauma, and neurodegenerative disorders. Dr. Ellingson has been a co-author on more than 100 peer-reviewed original research articles relating to neuroimaging has extensive experience performing data management and imaging analyses in multicenter clinical trials for brain cancer and other neuropathologies.

Elaine Hsiao, PhD
Assistant Professor, Department of Integrative Biology and Physiology, De Logi Chair in Biological Sciences, Division of Digestive Diseases, Department of Medicine, David Geffen School of Medicine at UCLA

Dr. Elaine Y. Hsiao is an Assistant Professor in the Department of Integrative Biology & Physiology and in the Department of Medicine at UCLA, where she leads a laboratory studying fundamental interactions between the microbiome, brain and behavior, and their applications to neurological disorders. Her previous work on neuroimmune contributions to neurodevelopment and behavior led to the finding that postnatal modification of the commensal microbiota improves gastrointestinal and behavioral symptoms in mouse models of genetic and environmental risk factors for autism. In addition, her laboratory identified select bacteria from the healthy human microbiome that promote host serotonin biosynthesis in the gut. Inspired by this interplay between the microbiota

and nervous system, the Hsiao laboratory is mining the human microbiota for microbial modulators of host neuroactive molecules, investigating the impact of microbiota-immune system interactions on neurodevelopment and examining the microbiome as an interface between gene-environment interactions in neurological diseases. Elaine received her Ph.D. in Neurobiology from Caltech, and her B.S. in Microbiology, Immunology and Molecular Genetics form UCLA.

Jonathan Jacobs, MD, PhD
Clinical Instructor, Division of Digestive Diseases, David Geffen School of Medicine at UCLA

Dr. Jonathan Jacobs received a B.A. in Biochemistry from Harvard University and a M.D. from Harvard Medical School. During this time he trained in the laboratory of Diane Mathis and Christophe Benoist, where he investigated the immunologic mechanisms of an autoantibody-mediated model of arthritis. This research was supported by a fellowship from the Howard Hughes Medical Institute and resulted in three first-author publications, including one in Proceedings of the National Academy of Science. He completed a residency in internal medicine at Stanford University then joined UCLA as a gastroenterology fellow in 2010. He pursued additional research training at UCLA through the Specialty Training and Advanced Research program under the mentorship of Jonathan Braun. He was awarded a Ph.D. in Cellular and Molecular Pathology in 2015 for his work on the interactions of the mucosal immune system and the intestinal microbiome in inflammatory bowel disease (IBD). Afterwards, he joined the UCLA Division of Digestive Diseases faculty as a Clinical Instructor. His ongoing projects employ in vivo models and multi'omics analysis of IBD cohorts to define the role of IBD-associated genes in shaping the intestinal microbiome and to identify microbial products that promote IBD.

Swapna Joshi, PhD
Assistant Project Scientist, Oppenheimer Center for Neurobiology of Stress, Division of Digestive Diseases, David Geffen School of Medicine at UCLA

Dr. Swapna Joshi is an Assistant Project Scientist at center for neurobiology of stress at UCLA. Swapna received her Ph.D. from Center for Cellular and Molecular Biology, India. Her research interests include bioinformatic analysis and integration of genomic, transcriptomic, epigenetic and microbial data in gastrointestinal disorders including irritable bowel syndrome (IBS). Early life stress is associated with an increased vulnerability toward developing IBS. Since DNA methylation is an interface between the dynamic environment and the fixed genome, Swapna's research interests include exploring the DNA methylation differences between IBS patients and healthy controls. Additionally, since changes in microbial composition have been associated with IBS, her current work is focused on understanding the role of microbiome and colonic mucosal microRNAs in the pathogenesis of IBS. She has published more than 24 articles in peer-reviewed high impact journals.

Jason Kutch, PhD
Assistant Professor, Division of Biokinesiology and Physical Therapy, University of Southern California

Jason J. Kutch, PhD, is an assistant professor in the Division of Biokinesiology and Physical Therapy at the University of Southern California. He teaches neuroscience in the USC Doctor of Physical Therapy program, and is the director of the Applied Mathematical Physiology Laboratory (AMPL) at USC. He is also a board member of the International Pelvic Pain Society.
Dr. Kutch's work focuses on revealing neural mechanisms of pelvic floor muscle control, engineering non-invasive systems to study human motor function, and better understanding neuromuscular disorders. He is a co-investigator in the NIH-funded Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network, with a particular focus on understanding brain network mechanisms of altered pelvic floor muscle control in individuals with chronic pelvic pain.

Jennifer Labus, PhD
Director, Neuroimaging and Bioinformatics Core – Oppenheimer Center for Neurobiology of Stress, Division of Digestive diseases, David Geffen School of Medicine at UCLA

Dr. Jennifer S. Labus is an Associate Professor in the David GeffenSchool of Medicine at University of California, Los Angeles. She is an investigator and Director for the Neuroimaging and Bioinformatics Core in the

Oppenheimer Family Center for Neurobiology of Stress at UCLA. Her research is focused on the interface of stress, pain and emotions and its influence on the role of dysregulation in the pathophysiology of common chronic pain disorders. She has unique expertise in applying advanced statistical and computational technologies to analyze multimodal brain imaging data. She has made seminal contributions to mapping neural networks underlying visceral pain. Dr. Labus' current research focus lies in applying a biological system based approach using bioinformatics, network analyses, supervised and unsupervised machine learning tools to integrate multimodal brain imaging data with other large scale biological data sets including genetics and metabolomics. This research provides the means to integrate and decipher large amounts of multivariate neuroimaging data to subgroup patients based on objective biological markers, and characterize central nervous system alterations for further pathophysiological investigations targeting treatment of chronic pain and obesity. She has been the recipient of a K08 Career Development award, Effective connectivity of central response in irritable bowel disorder, from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) as well as a RO3 award examining the role of altered attention and emotional arousal networks in IBS. Recently, acting as lead Co-Primary investigator she was awarded R01 funding by the National Institute of Childhood Health and Human Development (NICHD) to use brain imaging data, along with genetic, physiological and biological data, to extensively phenotype women with vulvodynia. Dr. Labus is a co-investigator on several NIH funded grants, international research collaborations, and is actively involved in mentoring graduate students and postdoctoral fellows. As a result of her work she was awarded the Master's Award in Gastroenterology in 2010 for her outstanding achievements in Basic and Clinical Digestive Sciences. Dr. Labus was also the recipient of the American College of Neuropsychopharmacolgy Travel Award in 2013.

Emeran Mayer, MD, PhD
Director, Oppenheimer Center for Neurobiology of Stress; Co-Director, CURE: Digestive Diseases Research Center; Division of Digestive Diseases, David Geffen School of Medicine at UCLA

Dr. Mayer has a career long interest in clinical and neurobiological aspects of how the digestive system and the nervous system interact in health and disease, and his work has been continuously supported by the NIH. He has published over 300 peer reviewed articles, including 90 chapters and reviews, co-edited four books, and organized several interdisciplinary symposia in the area of visceral pain and mind body interactions. He has made seminal contributions to the characterization of physiologic alterations in patients with various chronic pain disorders, such as irritable bowel syndrome (IBS), as well as on pharmacological and non-pharmacological treatment approaches to these conditions. He is principal investigator on several grants from the National Institutes of Health including a NIDDK/ORWH funded center grant on sex-related differences in brain gut interactions, a NIDDK funded consortium grant (Multidisciplinary Approaches to Pelvic Pain, MAPP) in which he also heads a multisite neuroimaging core, and on a NIDDK funded RO1 grant on the role of the immune system and the gut microbiome on brain signatures. His research efforts during the past few years have focused on several new areas of brain gut interactions, in particular on the role of the gut microbiota and their metabolites in influencing brain structure and function, and associated behavior, and on the role of food addiction in obesity.

Jeffrey F. Miller, PhD
Director, California NanoSystems Institute, Fred Kavli Chair, NanoSystems Sciences, UCLA

Jeff F. Miller, PhD, studies molecular mechanisms of bacterial pathogenesis and the evolution of functional diversity in bacteria and phage. He received his bachelor's degree in Chemistry from Case Western Reserve University and his Ph.D. in Molecular Biology from Tufts University School of Medicine. After postdoctoral training with Dr. Stanley Falkow at Stanford, he joined the faculty at UCLA in 1990. From 2002-2014 he held the M. Philip Davis Chair in Microbiology and Immunology and served as Chairman of the Department of Microbiology, Immunology and Molecular Genetics. In November, 2014, he was appointed as the Fred Kavli Endowed Chair in NanoSystems Sciences and Director of the California NanoSystems Institute at UCLA. In 2004, Dr. Miller co-founded AvidBiotics Corp., a biotherapeutics company in South San Francisco. In 2009 he was appointed by the Secretary of Health and Human Services to serve on the National Science Advisory Board for Biosecurity and he is a voting member of the Board. From 2008-2010 he was Chair of the General Meeting of the American Society for Microbiology (ASM), and from 2012-2014 he served as President of ASM, which represents 40,000 members in the US and abroad. Dr. Miller is a former Pew Scholar in the Biomedical Sciences, a member of the American Academy of Microbiology, a fellow of the American Association for the Advancement of Science, and in April, 2015 he was elected to membership in the National Academy of Sciences.

Nabila Moussaoui, PhD
Post-Doctoral Fellow, Division of Digestive Diseases, David Geffen School of Medicine at UCLA

I was born in 1987 at Saint -Gaudens close to Toulouse in France. I completed my PhD at the University of Paul Sabatier (Toulouse, FR) in 2014 in the Neurogastroenterology & Nutrition team , Institut National de la Recherche Agronomique , Toulouse, France. I studied the effects of a single episode of maternal separation on the integrity of the intestinal barrier and the hepatic transcriptome in the newborn. In January 2015, I started my postdoc fellow position at the Veterans Affairs Campus under the supervision of Professor Taché in the department Medicine-Digestive Diseases on research involving experimental stress model such as Limited Nesting stress to characterize the pup's physiology in term of intestinal barrier in relation to the HPA axis. I am also involved in experimental studies on the brain mechanisms of postnatal stress-induced alterations of visceral sensitivity with a focus on the loss of acute stress-related visceral analgesia.

Bruce Naliboff, PhD
Research Professor, Departments of Medicine and Psychiatry and Biobehavioral Sciences; Director, Pain Research Program - Oppenheimer Center for Neurobiology of Stress Division of Digestive Diseases, David Geffen School of Medicine at UCLA

Dr. Naliboff received his PhD in Clinical Psychology from Bowling Green State University in Ohio and interned at the UCLA Neuropsychiatric Institute. During his tenure at UCLA and the VA he has served as senior psychologist in the UCLA and VA Pain Management programs and Health Psychology Consultation services as well as a VA Career Scientist. Dr. Naliboff's VA and NIH funded research has focused on psychophysiological mechanisms of stress and pain and includes studies of stress effects on the immune system, glucose regulation in diabetes, and cardiovascular variables. In the area of pain, he has utilized experimental pain procedures to study perceptual processes in chronic pain states such as chronic back pain, headache, and visceral pain. He has also studied psychosocial and personality variables in chronic pain and especially their impact on treatment choice and outcome. His work in functional gastrointestinal disorders and irritable bowel syndrome (IBS) include perceptual, autonomic, and brain imaging studies of visceral sensation, and the role of psychosocial variables in the presentation, course and treatment of IBS. A major emphasis of Dr. Naliboff's recent work is the development and evaluation of mind body therapies for both visceral and somatic pain. This includes NIH funded studies of a novel cognitive behavioral therapy for IBS, discovery of brain biomarkers associated with training in mindful meditation for IBS and post-traumatic headache, and pilot studies of Yoga for both visceral and somatic pain. He has served as a consulting editor for numerous scientific publications in psychology and medicine and on national and international committees as a grant reviewer and program consultant.

Larissa V. Rodriguez, MD
Professor of Urology, Keck School of Medicine, University of Southern California

Dr. Larissa Rodríguez was appointed Associate Provost for Faculty and Student Initiatives in Health and STEM on July 1, 2015. She is a Professor of Urology and the director of Female Pelvic Medicine and Reconstructive Surgery (FPMRS) at Keck Medicine of USC – Beverly Hills clinic. She is also vice chair of academics for the USC Institute of Urology and director of the FPMRS Fellowship at the Keck School of Medicine.

Dr. Rodríguez is an established surgeon-scientist with extensive expertise in the field of female pelvic and reconstructive surgery in urology. An awarded researcher, she is nationally and internationally recognized for her work in the field of stem cell research and tissue engineering of the urinary tract and in animal models of lower urinary tract dysfunction. She has focused some of her clinical and outcomes research on the etiology and treatment of urinary incontinence and vaginal prolapse, and has developed patented techniques and innovative surgical strategies to provide treatment in areas such as stress incontinence and reconstruction of the bladder and lower urinary tract. She has served on numerous NIH study sections and national and international organizations.

Claudia Sanmiguel, MD
Director, Ingestive Behavior and Obesity Research Program - Oppenheimer Center for Neurobiology of Stress
Division of Digestive Diseases, David Geffen School of Medicine at UCLA

Claudia Sanmiguel is the Ingestive Behaviors and Obesity Program (IBOP) director at the Gail and Gerald Oppenheimer Family Center for Neurobiology of Stress (CNS) and she is a clinical instructor at the UCLA Digestive Diseases Division. She was born in Bogota, Colombia where she studied Medicine at the Pontificia Universidad Javeriana and specialized in Internal Medicine and Gastroenterology. In pursue her interests in GI motility and the role of GI signals in weight control she conducted translational and clinical research at the University of Alberta, Canada, at the Cleveland Clinic in Ohio and at the Cedars Sinai Medical Center in Los Angeles, where she explored the use of pacemakers and electrical stimulators for the treatment of diverse GI motility disorders as well as their role in the obesity and obesity related diabetes mellitus treatment. She also studied the role of gastric electromechanical signals related to eating behavior and satiety. As part of pursuing a research career in United States, she completed her residency in Internal Medicine at Cedars-Sinai Medical Center and trained in Gastroenterology at the University of California Los Angeles. Her current research interests are focused on the role of the gut-brain axis in the regulation of eating behaviors and weight control in health and in disease, with emphasis in obesity. She has published several papers in well known GI, neurosciences and bioengineering journals and presented her research results in North American and International meetings. She currently has NIH funding for a study on the role of changes in brain activity and control of eating behaviors in weight loss after bariatric surgery and the mechanisms underlying those changes. She is also testing the role of neuromodulation on the control of eating behaviors.

Kirsten Tillisch, MD
Director, Mind Body Research Program - Oppenheimer Center for Neurobiology of Stress Division of Digestive Diseases, David Geffen School of Medicine at UCLA

Dr. Kirsten Tillisch is an Associate Professor of Medicine in the Division of Digestive Diseases at the David Geffen School of Medicine at UCLA and the Chief of Integrative Medicine at the Greater Los Angeles VA. Dr. Tillisch's clinical interests include the promotion of non-pharmacological and integrative therapies for chronic disease and wellness, functional bowel disorders, and chronic pain. Her research interests include brain-gut and microbiome-gut-brain interactions, the effects of non-pharmacological therapies on chronic disease, and pharmacological treatment of irritable bowel syndrome. She is a member of the Rome IV Committee on Functional Abdominal Pain. She has been an NIH funded researcher since 2006, utilizing neuroimaging techniques to study the physiology of brain gut interactions. She currently studies the central effects of Mindfulness Based Stress Reduction on symptoms of irritable bowel syndrome and post traumatic headache. Her recent research projects also include evaluation of the role of gut microbiota modulation on emotional processing in the brain, the benefits of Mindfulness Based Stress Reduction on mood in Veterans, and assessment of hypnosis effects on the gut and brain in irritable bowel syndrome. She is a member of the Neuroimaging Program of the Gail and Gerald Oppenheimer Family Center for Neurobiology of Stress.

Lixin Wang, MD, PhD
Researcher, Division of Digestive Diseases, Department of Medicine, David Geffen School of Medicine at UCLA

Dr. Lixin Wang received her MD and PhD in neuroanatomy at Beijing University Medical School in China, where she taught human anatomy and neuroanatomy to medical students and graduate students. She did 2.5 years research on human skin innervation in the Karolinska Institute in Stockholm, Sweden. She joined the Division of Digestive Diseases, Department of Medicine at UCLA in 1993. Her experimental research focus is on brain-gut interaction in regulation to feeding behavior and gastrointestinal transit under stress, inflammation, visceral obesity and in animal models of Parkinson's disease. Dr. Wang is the PI of a VA-founded project on treatment for non-motor disorders of Parkinson's disease in animal models. She is also a key investigator in several projects of Dr. Taché funded by NIHDDK and VA and 2 center grants by NIHDDK in Digestive Disease Division.

Zhuo Wang, PhD
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Zhuo Wang earned his BS degree in Biology t the University of Science and Technology of China, and PhD in Neurobiology at USC. He then received postdoctoral training at the VA Greater Los Angeles Healthcare System and the Center for Neurobiology of Stress at UCLA, before joining USC as a research faculty. Dr. Wang's research focuses on functional brain mapping and functional connectivity analysis in rodent models of visceral pain, with an emphasis on understanding the brain mechanisms underlying sex differences and effects of chronic stress. His main approach is autoradiographic blood flow mapping in awake, nonrestrained rodents, performed in parallel with other physiological and behavioral measurements. His research interests also include understanding the causal interactions in the resting-state brain network, and understanding circuit-level neuropathology of neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's diseases, in search for new treatment targets.

Abstracts of Posters
Basic and Translational

1.
Identification and Characterization of Colonic-exosomes: Micro-RNA Mediated Horizontal Gene Transfer within the Colonic Epithelia *In Vitro* and *In Vivo*

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Background and Aims: Intercellular crosstalk directed by exosomes is gaining increasing attention. Intestinal epithelial cell exosomes and their genetic cargo have been studied extensively in colon cancer and gut-microbiome intercommunication. The differential biogenesis of colonic exosomes during inflammatory bowel disease (IBD) and their role in IBD-driven intercellular crosstalk, however, is not known. We have previously shown that proinflammatory signaling alters the total, cytoplasmic microRNA expression profile in human intestinal epithelial NCM460 cells and in colon samples from IBD patients. These changes may be translated in differential sorting of intestinal epithelium exosomal miRNA cargo. We hypothesized that during IBD, colonic epithelial cells secrete exosomes enriched in specific miRNAs, regulating key signaling pathways within the gut. **Methods:** We used human blood serum from control or ulcerative colitis (UC) patients (n=5 per group) as well as culture media from NCM460 cells treated with a proinflammatory cytokine cocktail (PCC: IL-6, IL-8, INF-γ and TNF-α, 10 μg/ml, 6 h, n=6). Exosomes were isolated by sequential ultracentrifugation for all in vitro and in vivo models. We assessed relative exosome biogenesis by western blot analysis using an antibody against the intestinal epithelial cell specific marker A33. Exosomes isolated from NCM460 cell culture media following PCC treatment were used to assess 1) exosomal microRNA cargo via qPCR, 2) Exo-Glow-labeled exosome re-absorption by NCM460s and 3) cell proliferation and migration of NCM460 cells following exposure to isolated exosomes. Intracolonic infusion of exosomes isolated from mouse colonic epithelial cells collected from control or an experimental colitis (TNBS, 5mg/kg, 48 h) mouse model into the colon of recipient, control or sub-colitic (TNBS, 2mg/kg, 48 h) mice was used to asses in vivo exosome-induced inflammatory signaling (n=5 mice per group). **Results:** Intestinal exosomes were increased in UC blood serum compared to control serum (p=0.0051) as well as in NCM460 cell culture media following exposure to PCC (p=0.0057) while their miRNA cargo profile was significantly altered in both conditions. Exo-Glow-labeled, NCM460-secreted exosomes were readily re-absorbed by recipient NCM460 cells observed by increased cytoplasmic green fluorescence of recipient cells. Exosomes produced by 1) NCM460 cells exposed to PCC or 2) colonic epithelium collected from mice with experimental colitis (TNBS, 5mg/kg, 48 h), promoted a) NCM460 cells proliferation and migration or b) IL-6 (p=0.0336) and IFN-γ (p=0.0001) mRNA expression in colonic epithelia of sub-colitic (TNBS, 2mg/kg, 48 h) mice, compared to infusion of exosomes from control mice. **Conclusions:** This study provides the first insight into the use of exosomes as molecular markers for diagnostic and therapeutic intervention for IBD.

2.
MiR-31-3p Is Involved in Substance P (SP)-associated Inflammation in Human Colonic Epithelial Cells and Experimental Colitis.

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Background and Aims: MicroRNAs (miRs) are small RNAs that negatively modulate the expression of various genes. We have reported that miR-31-3p is induced by SP stimulation in NCM460 colonocytes overexpressing the SP, neurokinin-1 receptor (NCM460-NK-1R) (Fang K et al, CMGH In press). We also reported during DDW 2015 that mucosal expression of miR-31-3p is increased during experimental colitis and ulcerative colitis colon tissue and modulates RhoA expression in NCM460-NK-1R cells in response to SP. However, the role of miR-31-3p during colitis has never been investigated. Our aim was to determine the importance of miR-31-3p in inflammatory signaling and experimental colitis. **Methods:** NCM460-NK-1R cells were transfected with anti-miR-31-3p or anti-miR-control using siPORT NeoFX (Ambion). Constitutively active RhoA was expressed in vitro

using an adenoviral system (Cell Biolabs). Acute mouse colitis was induced by intracolonic TNBS administration or addition of DSS in the drinking water, respectively. The role of miR-31-3p was examined by administrating antisense (as)-miR-31-3p or as-miR-control intracolonicaly into DSS-exposed mice, and the in vivo function of miR-31-3p during the healing phase of colitis by administrating (as)-miR-31-3p or as-miR-control intracolonicaly during the 10 days of healing following 5 days of DSS treatment. **Results:** Bioinformatics analysis indicated that RhoA was a possible downstream target of miR-31-3p. Indeed, transfection with a miR-31-3p mimic decreased the protein level and activity of RhoA in NCM460-NK-1R cells following SP exposure. Expression of constitutively active RhoA also increased SP-induced CCL2, IL6, TNFα, and CXCL10 expression in NCM460-NK-1R cells (n=3, $p<0.05$), while miR-31-3p silencing increased CCL2 (by 54%) and IL6 (by 2.1 fold) mRNA (n=3, $p<0.05$). Overexpression of miR-31-3p inhibited TNFα, CCL2, IL6 and IL1β mRNA expression induced by a cytokine-cocktail (10μg/ml of TNFα, IFN-γ, IL6 and IL8) in NCM460 cells (n=3, $p<0.05$). NK-1R deficient TNBS-exposed mice had reduced miR-31-3p levels, compared to wild-type colitic mice, suggesting a SP-dependent response. Intracolonic as-miR-31-3p administration increased colitis histopathology score as well as colonic expression of CCL2, TNFα and Cxcl10 mRNA (n=8, $p<0.05$) 5 days after DSS administration, but had no effect on the healing phase of DSS-induced colitis. **Conclusions:** This is the first demonstration that the SP-induced miR-31-3p regulates inflammatory gene expression and colitis, representing a novel anti-inflammatory regulator for colitis and IBD.

Supported by NIDDK RO-1 DK 47343 (CP), a CURE: DDRC P30 DK 41301 Pilot and Feasibility study (KF), T32 DK07180-40 (DP), and by a Research Fellowship from the Crohn's and Colitis Foundation (IKML).

3.

Mesenteric Preadipocytes from Crohn's Disease Patients Exhibit Differential mRNA Expression Patterns and Induce Protective Responses in Colonic Epithelial Cells and Mice with Colitis

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Background and Aims: Mesenteric adipose tissue hyperplasia is a hallmark of Crohn's Disease (CD). Recently, we have shown that conditioned media from preadipocytes isolated from CD, Ulcerative Colitis (UC) and control patients alter expression of inflammatory mediators in human colonic epithelial cells in a disease-dependent manner. The goal of this study was to examine the mRNA expression profiles of human mesenteric preadipocytes and assess the effects of preadipocyte-derived mediators on intestinal epithelial cell signaling *in vitro* and experimental colitis *in vivo*. **Methods:** Preadipocytes were isolated from mesenteric fat of control and CD patients and conditioned media and total RNA collected from cultured cells. Microarray profiling of mRNA transcripts (6/group) was performed in isolated preadipocytes and human colonic epithelial NCM460 cells treated with conditioned media (8-11/group). Network analysis was performed using Ingenuity Pathway Analysis (IPA). NCM460 proliferation was assessed using the xCelligence platform. Experimental colitis was induced in C57BL/6J mice by administering dextran sodium sulfate (DSS) in their drinking water (3.5% w/v) for 5 days (8/group). Mice received daily intracolonic injections (0.1 ml) of vehicle or conditioned media from control or CD preadipocytes. Separate groups of mice were switched to water for an additional 10 days and received injections on days 6-15 (24 mice/group; 3 patients/group). RNA was extracted from full-thickness sections of distal colon for qPCR. **Results:** CD-derived preadipocytes had differential mRNA expression compared to controls, and network analysis predicted activation of pathways promoting phagocytosis of bacteria, as well as cell growth and proliferation. A central regulator in the highest predicted network was caspase 8 ($p<0.05$). Media from CD patients increased cell proliferation and SERPINE-1 mRNA expression ($p<0.05$) in NCM460 cells compared to control patients, and network analysis predicted alterations in injury and inflammation pathways. DSS-treated mice injected daily with media from CD preadipocytes had decreased severity of colitis as indicated by histological scores (2.9±1.00 vs 6.3±0.73, $p<0.05$) and decreased CXCL1 mRNA expression compared to controls during acute colitis ($p<0.01$), while injections of CD-derived media decreased CCL2 ($p<0.05$) mRNA compared to control media during recovery from DSS colitis. **Conclusions:** Preadipocytes isolated from control and CD patients show differential disease-dependent inflammatory responses and can alter colonic epithelial cell signaling in vitro and proinflammatory cytokine expression in DSS-treated mice *in vivo*. We suggest that mesenteric adipose tissue derived mediators may participate in the pathophysiology of CD by promoting colonocyte proliferation and the resolution of inflammation.

4.

Early Life Stress Elicits Visceral Hyperalgesia and Functional Reorganization of Pain Circuits in Adult Rats

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Early life stress (ELS) is a risk factor for developing functional gastrointestinal disorders, and has been proposed to be related to a central amplification of sensory input and resultant visceral hyperalgesia. We sought to characterize ELS-related changes in functional brain responses during acute noxious visceral stimulation. Neonatal rats (males/females) were exposed to limited bedding (ELS) or standard bedding (controls) on postnatal days 2-9. Age 10-11 weeks, animals were implanted with venous cannulas and transmitters for abdominal electromyography (EMG). Cerebral blood flow (rCBF) was mapped during colorectal distension (CRD) using [^{14}C]-iodoantipyrine autoradiography, and analyzed in three-dimensionally reconstructed brains by statistical parametric mapping and functional connectivity. EMG responses to CRD were increased after ELS, with no evidence of a sex difference. ELS rats compared to controls showed a greater significant positive correlation of EMG with amygdalar rCBF. Factorial analysis revealed a significant main effect of 'ELS' on functional activation of nodes within the pain pathway (somatosensory, insular, cingulate and prefrontal cortices, locus coeruleus/lateral parabrachial n. [LC/LPB], periaqueductal gray, sensory thalamus), as well as in the amygdala, hippocampus and hypothalamus. In addition, ELS resulted in an increase in the number of significant functional connections (i.e. degree centrality) between regions within the pain circuit, including the amygdala, LC/LPB, insula, anterior ventral cingulate, posterior cingulate (retrosplenium), and stria terminalis, with decreases noted in the sensory thalamus and the hippocampus. Sex differences in rCBF were less broadly expressed, with significant differences noted at the level of the cortex, amygdala, dorsal hippocampus, raphe, sensory thalamus, and caudate-putamen. ELS showed a sexually dimorphic effect ('Sex x ELS' interaction) at the LC/LPB complex, globus pallidus, hypothalamus, raphe, septum, caudate-putamen and cerebellum. Our results suggest that ELS alters functional activation of the thalamo-cortico-amydala pathway, as well as the emotional-arousal network (amygdala, locus coeruleus), with evidence that ELS may additionally show sexually dimorphic effects on brain function.

5.

Variability of Psychosocial Stress Responses in Vervet Monkey Model

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Psychological stress is a key factor in common chronic diseases, particularly cardiovascular, metabolic, and neuropsychiatric disorders. Human stress studies suffer from the infeasibility to control or even account for environmental factors, such as preexisting stress exposures, and the infeasibility of longitudinal and sometimes invasive sampling. These obstacles can be circumvented in non-human primates, which more precisely reflect human brain circuitry, behavior, physiology than rodents and other model organisms. Here, we present a vervet monkey model for systems biology studies of psychosocial stress. The vervet model of response to psychosocial stress is being developed by leveraging procedures routinely employed at the St. Kitts Biomedical Research Foundation (SKBRF), in which newly trapped vervets are temporarily housed individually while assessed for carriage of infectious agents. This quarantine represents a major psychosocial stressor and provides an opportunity for physiological, behavioral, and cognitive assessments. Our preliminary results from 15 juvenile male vervets subjected to the 28-day quarantine demonstrate that this setting evokes measurable and significant stress responses in vervets, specifically in hypothalamic–pituitary–adrenal (HPA) activity, glucose metabolism, and immune system function. First, a significant increase in hair cortisol between day 0 and day 28 (108% increase, p-value=0.0083, paired t-test) was observed; this is consistent with chronic HPA activation during psychosocial stress. Second, assessments of plasma lactate concentrations, a biomarker of psychosocial stress in humans, showed high fasting lactate levels in the blood in day 3 (3.4 mmol/l) and a subsequent gradual

decrease to 1.1 mmol/l in day 28. This is similar to normal lactate levels in humans (68% decrease). It is consistent with the rise of lactate concentrations in response to acute stressor and subsequent normalization once animals habituate to the stressor. Third, nine cytokines (IL1B, RANTES, HGF, INFg, MDC, MIF, MCP1, IL-1RA, and eotaxin) show a coordinated gradual decrease in response to the stressor. This reaches a maximum of stress exposure in day 14, consistent with the suppression of the immune system by chronic stress, resulting from immunosuppressive effects of glucocorticoids. After day 14, average cytokine levels (except eotaxin and IL-1RA) begin recovering toward baseline, suggesting a rebound of immune functions due to habituation to the stressor. The lactate and immune responses show a significant between-monkey variability, suggesting genetic contributions to these phenotypes. Behavioral studies and assessments of monoamine neurotransmitter levels are underway. This proof-of-principle study shows that a quarantine setting represents a stressor with significant consequences in neuroendocrine, immune, and glycemic functions

6.

Functional Pathways Associated with Differentially Expressed Colonic Mucosal microRNA and mRNA in Irritable Bowel Syndrome

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Background: Irritable bowel syndrome (IBS) is a stress-sensitive disorder characterized by chronic abdominal pain and diarrhea and/or constipation. Peripheral mechanisms contributing to visceral hypersensitivity, altered gut secretion and motility are incompletely understood. MicroRNAs (miRNAs) are small RNAs capable of regulating mRNA levels. MiRNA-mRNA functional pathways have recently been identified in a water avoidance stress (WAS) rat model of IBS (Bradesi S. et al., PLoS One 2015). **Aim:** To institute an integrative pathway analysis approach of differentially expressed miRNA-mRNA target pairs in the colonic mucosa in IBS compared to healthy controls (HCs). **Methods:** Female and male Rome III + IBS and age and sex matched HCs underwent sigmoidoscopy with sigmoid colon biopsies, from which total RNA was extracted. MiRNAs were measured using nCounter miRNA assay from NanoString. Gene expression (mRNA) was measured using ArrayStar microarrays in a subset of IBS patients and HCs. Differential expression was identified using Student's t-tests. A p-value <0.05 was considered significant. Validated miRNA targets were obtained from miRtarBase (a database for experimentally validated miRNA-target interactions). Pathways/Gene Ontology (GO) terms were identified using Enrichr (a gene-list enrichment analysis tool). **Results:** 29 IBS (55% F, mean age 34 yrs, 14 IBS-D and 15 IBS-C) and 15 HCs (47% F, mean age 34 yrs) participated. Sixteen miRNAs were significantly different between IBS and HCs (p<0.05). In the subset of 20 IBS patients (10 IBS-D, 10 IBS-C) and 10 HCs (50% F in both groups), there were 2075 differentially expressed mRNAs (p<0.05). Of these, we identified 308 that overlapped with validated targets of differentially expressed miRNAs and 137 miRNA-mRNA pairs that were deregulated in opposite directions (i.e., high miRNA-low mRNA and low miRNA-high mRNA, Figure 1). Top 5 pathways associated with the mRNAs from 137 miRNA-mRNA pairs are shown in Table 1. **Conclusions:** Using an integrative approach, we identified deregulation of validated mRNA targets of differentially expressed miRNAs in the colonic mucosa associated with nociceptive and neuroinflammatory pathways. Janus kinase (JAK) and Signal Transducer and Activator of Transcription 3 (STAT3) signaling pathways have been associated with neuroinflammation and neuropathic pain and found to be upregulated in lumbar spine in WAS rats with visceral hyperalgesia. Similarly, fibroblast growth factor receptor (FGFR) signaling mediates axon-glial interaction in the peripheral sensory pain pathways. Nerve growth factor (NGF) signaling has been implicated in visceral sensitivity via expression of NGF and its receptor (tyrosine kinase, TrkA) in neuronal and non-neuronal tissues of the gut. These findings provide new potential pathophysiologic insights and potential drug development targets in IBS.

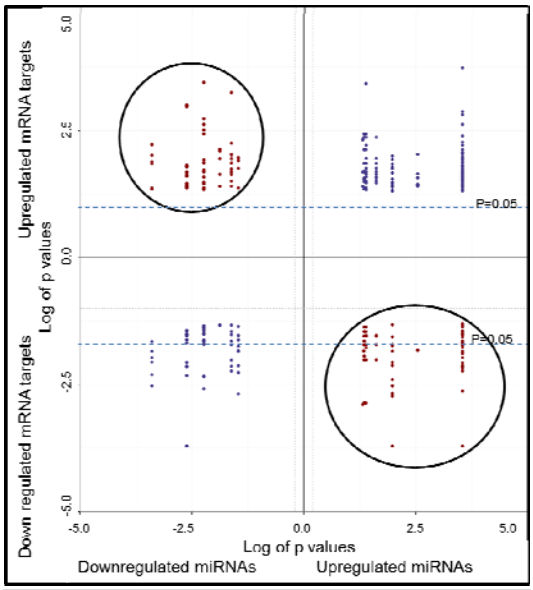

Figure 1: Scatterplot of differentially egulated miRNA-target mRNA pairs.

Table1: Gene Ontology terms associated with the differentially expressed miRNA - target mRNA pairs.

Term	P	FDR	Genes
Cellular responses to stress	0.001	0.286	RB1;UBB;STAT3;HMGA1;TNRC6A;MAP4K4;VEGFA
Cellular Senescence	0.001	0.286	RB1;UBB;STAT3;HMGA1;TNRC6A;MAP4K4
Signaling by FGFR in disease	0.006	0.345	UBB;SHC1;STAT3;FOXO3;TNRC6A
Signaling by NGF	0.011	0.345	NTRK2;UBB;SHC1;STAT3;FOXO3;TNRC6A
NGF signaling via TRKA from the plasma membrane	0.012	0.345	NTRK2;SHC1;STAT3;FOXO3;TNRC6A

FGFR, fibroblast growth factor receptor; NGF, nerve growth factor; TRKA, tyrosine kinase A.

7.

FFA3 Activation Inhibits Nicotine-Induced Secretion and Motility via Enteric Nervous Reflex in Rat Proximal Colon

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Background and Aim: Short-chain fatty acids (SCFA) are microbial fermentation products that are absorbed from the colon. Although luminal SCFA stimulate colonic secretion and contraction, we have recently reported that activation of an SCFA receptor FFA3, expressed in cholinergic nerves, suppresses cholinergic-mediated transepithelial anion secretion. We further investigated how activation of neurally-expressed FFA3 affects colonic motility. **Methods:** The selective FFA3 agonists (N-[2-methylphenyl]-[4-furan-3-yl]-2-methyl-5-oxo-1,4,5,6,7,8-hexahydro-quinoline-3-carboxamide; MQC) and AR420626 were used to activate FFA3 expressed in the enteric neurons. Short-circuit current (I_{sc}) was measured to determine anion secretion in Ussing chamber. Circular muscle contractions were measured with isolated muscle strips without mucosa and submucosa. The effect of FFA3 agonists on defecation *in vivo* was investigated with an established exogenous serotonin-induced defecation model. **Results:** In Ussing chambered mucosa-submucosa preparations, pretreatment with MQC or AR420626 suppressed the I_{sc} increases induced by carbachol (CCh) or nicotine by 65%, but did not affect the response to bethanechol or forskolin. The inhibitory effect of FFA3 activation on CCh-evoked I_{sc} was reversed by pretreatment with the $G_{i/o}$ inhibitor pertussis toxin. Circular muscle strips exhibited periodic spontaneous contractions, which frequency was enhanced by the NO synthase inhibitor L-NAME, indomethacin, or AR420626, but not by MQC. The addition of nicotine (10 μM) transiently relaxed the muscle, inhibited by TTX or L-NAME. High concentration of nicotine (100 μM) induced large amplitude contractions that were not altered by TTX, L-NAME, or indomethacin. Pretreatment with FFA3 agonists inhibited the nicotine-induced relaxation and contraction, but had no effect on bethanechol-induced contractions. The effect of AR420626 was more potent than that of MQC. Nicotine-evoked contractions were abolished by AR420626 and reversed by pretreatment with pertussis toxin, suggesting that FFA3 activation suppresses nicotinic neural activity in the myenteric neurons, consistent with a FFA3-mediated secretory pathway. In conscious rats, serotonin (10 mg/kg, i.p.) treatment significantly increased the volume of fecal output, compared with the non-treated group, unaccompanied by diarrhea. Pretreatment with AR420626 (0.1 mg/kg, i.p.) significantly suppressed serotonin-induced fecal output. **Conclusion:** FFA3 agonists may be useful therapeutically for diarrheal disorders due to their highly targeted and localized anti-cholinergic effects.

Supported by VA Merit Review and NIH R01 DK54221

8.

Intracerebroventricular Corticotropin-Releasing Factor (CRF) at Low Doses Induces a CRF_1 Receptor-Mediated Visceral Analgesia in Male Rats through Recruitment of Brain Oxytocin

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Background: Psychological stress induces a naloxone-independent visceral analgesia to colorectal distension (CRD) in male rodents when monitored using a non-invasive method of visceromotor response (VMR) measurement based on manometry. Brain CRF mediates the stress and pain responses via differential

recruitment of CRF_1 (anxiogenic, pronociceptive) and CRF_2 (anxiolytic, antinociceptive) receptors. Stress and pain are intimately associated; central mediators released under stress conditions including opioids and oxytocin can attenuate stress and anxiety influences on nociceptive signaling. **Aims:** To determine if ICV CRF can reproduce the analgesic effect of psychological stress on VMR to CRD when monitored non-invasively, and whether CRF, opioids, and/or oxytocin receptors are involved in this visceral analgesia. **Methods:** Ten days before the experiment, adult male Sprague Dawley rats (250-300g, 2/cage, n=5-15) were surgically implanted with an intracerebroventricular (ICV) cannula. The VMR to graded phasic CRD (10, 20, 40, 60 mmHg, 20 sec duration, 4 min intervals) was monitored using manometry. A 1st CRD served as baseline, then after 1h of rest, rats were injected ICV with either CRF (30, 100, 300 ng, 1 μg, 3 μg and 5 μg/rat) or vehicle (saline, 10 μl). Five minutes after injection, a 2nd CRD was performed. In other sets of experiments, rats were pretreated with the following specific receptor antagonists: non-selective opioid, naloxone (SC, 1 mg/kg in 0.3 ml saline, 10 min), CRF_1/CRF_2, astressin-B (ICV, 30 μg/rat in 5 μl sterile water, 5 min), the selective CRF_2, $astressin_2$-B (ICV 10 μg/rat in 5 μl saline, 5 min), the oxytocin tocinoic acid (20 μg/rat in 5 μl saline, 5 min) or respective vehicles before ICV CRF (300 ng/rat, 5 μl). Results expressed in % of maximal basal VMR were analyzed using 2-way ANOVA and Bonferroni post-hoc test. **Results:** ICV CRF at 30 ng did not change the VMR compared to vehicle (n=11). At 100 (n=12) and 300 ng (n=10), ICV CRF reduced the VMR at 60 mmHg (-36.6±6.8% and -48.7±11.7% respectively, $p<0.001$) compared to baseline. ICV CRF analgesia disappeared at higher doses (1, 3 and 5 μg, n=6, 15 and 11, respectively). Pretreatment with astressin-B (n=9) and tocinoic acid (n=5) prevented the analgesic effect of ICV CRF (300 ng/rat) while pretreatment with naloxone (n=5) and $astressin_2$-B (n=6) had no effect. The receptor antagonists alone had no effect on basal VMR. **Conclusions:** In a noninvasive model of visceral pain, CRF ICV injected at low doses (100 and 300 ng) leads to a CRF receptor-mediated naloxone-independent visceral analgesia that may recruit downstream oxytocin pathway. These results also suggest a dual action of brain CRF: a visceral stress analgesia that could be abrogated at a high intensity of stress.

Supported by NIH DK-57238 (YT), 1K01DK088937 (ML), NIH-078676 (MM)

9.

Neonatal Limited Nesting Stress Influences Basal and Post-Stress Visceral Sensitivity in a Sex-Dependent Manner in Adult Wistar Rats

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Background: Early life adversity contributes to the development of stress-sensitive disorders such as irritable bowel syndrome (IBS) with higher occurrence in females. **Aim:** The limited nesting stress (LNS) model was recently validated as a model of fragmented and erratic maternal behavior in the context of an impoverish environment in rodents, but its influence on visceral sensitivity in adult rats under basal or stress conditions is not well known. **Methods:** Time-pregnant Wistar females (E15, Harlan) were used. From PND2 to PND9, dams were housed under control (direct contact bedding + 1 paper towel) or LNS conditions (wire bottom floor + ½ paper towel). Once adult, rats (9-14 wks, n=6-14) received a basal colorectal distension (CRD, 10, 20, 40, 60 mmHg, 20 sec, 4 min intervals) before being exposed to superimposed repeated water avoidance stress (rWAS, 10 days, 1h/day) or no stress. The visceromotor response (VMR) was monitored non-invasively using manometry. Additional CRDs were performed immediately (IMM), 24h and 72h after the last stress session. **Results:** Control and LNS female rats had similar baseline VMR, while LNS male rats exhibited a decrease in VMR at 40 mmHg vs control males ($p<0.05$). Females' VMR was higher than males at 60 mmHg in controls ($p<0.01$) and LNS ($p<0.0001$) rats. Repeated WAS induced visceral analgesia at 40 mmHg IMM and 40 and 60 mmHg 72h post stress in control males but only a tendency at 40 and 60 mmHg in control females. LNS-raised males lost their analgesic responses and developed visceral hyperalgesia at 60 mmHg IMM post rWAS, while LNS females presented a visceral analgesia at 60 mmHg IMM post rWAS. Repeated CRDs did not affect the VMR of control or LNS-raised rats. **Conclusions:** Our data show that neonatal LNS induces a basal visceral analgesia in adult Wistar male rats but not females and leads to a loss of superimposed stress-induced visceral analgesia and the development of stress-induced hyperalgesia in males exclusively. These findings suggest that early life events affect both the basal and a superimposed stress-related visceral pain response of rodents in a sex-dependent manner.

Supported by NIH P50-DK-64539, DK 57238 and K01DK088937

10.

Chronic Oral Exposure To Ultrafine Particulate Matter Alters The Cecal Microbiota Composition But Not Ileal Permeability in LDLR-Null Male Mice Fed A High Fat Diet

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Background: Chronic exposure to ambient ultrafine particulates (UFP, dp<180 nm) is a well-recognized risk factor for cardiopulmonary diseases in association with increased morbidity and mortality. Epidemiological studies revealed a link between air pollution exposure and gastrointestinal diseases, including inflammatory bowel disease (IBD). We recently found that chronic respiratory exposure to UFP promotes ileal villus shortening and inflammatory responses in mice. **Aim:** As inhaled pollutants such as UFP are cleared by mucociliary transport into the intestine and UFP contaminate both food and water supplies, we assessed whether the direct contribution of orally administered UFP resulted in ileal inflammatory responses in mice. **Methods:** UFP representing a mixture of pollution sources, including size fractionated fresh ambient particulate matter (PM) and PM generated by photochemical oxidation of primary organic vapors were collected near downtown Los Angeles, CA. Adult male *Ldlr*-null mice (84 days old, ~25 g) on a Western-type high-fat diet (21% milk fat, 0.2% cholesterol)(Teklad diet TD88137, USA) were gavaged 3 days/week with UFP (40 μg/mouse, n=12) or vehicle (100 μl saline, n=11) for 10 weeks. At week 9, mice were gavaged with 150 μl FITC–dextran 4kDa (FD4) (12 mg/mouse). After 4h, the concentration of FD4 in facial blood was read by fluorimeter to assess *in vivo* gut permeability. At week 10, after euthanasia, ileal samples were collected and mounted in Ussing chambers after seromuscular peeling to assess changes in epithelial resistance (TEER), secretion (Isc) and mucosal-to-serosal flux of FD4. Bacterial DNA was extracted from cecum and the V4 region of 16S rDNA was sequenced (MiSeq, Illumina) to determine bacterial composition. **Results:** *Ldlr*-null mice weight was similar in UFP and vehicle treated mice throughout the study. At week 9, UFP and vehicle-treated mice exhibited similar intestinal permeability to FD4 gavage (8.7±0.3 vs 9.5±0.5 μg/ml). At week 10, the ileal TEER was increased in UFP vs vehicle-treated mice (21±2 vs 14±2 Ω/cm^2, $p<0.05$), but the mucosal-to-serosal flux of FD4 and serosal FD4 concentration at 2h were not affected. The ileal Isc (167±36 vs. 131±43 $\mu A/cm^2$) and ΔIsc to carbachol (123±20 vs. 125±23 $\mu A/cm^2$) of UFP vs vehicle-treated mice were unchanged. Chronic UFP gavage reduced the numbers of *Lachnospiraceae* (negatively associated with colon cancer) and *Gloeobacterales* (anti-inflammatory and anti-oxidant properties) bacteria and increased the number of *Verrucomicrobiaceae* (intestinal mucus degradation) in the mice cecum. **Conclusions:** Orally administered UFP significantly altered the composition of the cecal microbiota, implicating mucosal protection to mitigate ileal epithelial dysfunction. These results suggest alternative systemic mechanisms underlying chronic ambient UFP exposure and intestinal inflammatory responses.

11.

The Trans-Golgi Network Protein Aftiphilin Regulates Intestinal Epithelial Permeability in Human Colonic Epithelial Cells

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Background and Aims: Aftiphilin (AFTPH) is localized in the trans-golgi network (TGN) and is involved in intracellular trafficking. We have recently found that AFTPH expression is downregulated in colonic epithelial cells in TNBS-induced mouse colitis and patients with ulcerative colitis (Law et al, Gut 2015). However, the role of AFTPH in epithelial cell signaling and colitis are unknown. Since epithelial cell permeability is an important feature of colitis and is related to TGN function, we examined the hypothesis that AFTPH may regulate epithelial cell permeability during colitis. **Methods:** AFTPH gene-silencing was performed by transfection of small interfering RNA (siRNA) against AFTPH (si-AFTPH) in human NCM460 colonic epithelial cells, while AFTPH overexpression in vitro was by achieved by transduction of lentivirus-expressing AFTPH. Cellular localization of E-cadherin (CDH1) and tight junction protein ZO1 in NCM460 cells was examined by immunocytochemistry (ICC). Epithelial

cell permeability was studied using Dextran (3 kMW and 10 kMW) conjugated with Alexa Fluor® 680 (Life Technologies) and trans-epithelial electric resistance (TEER) was measured with the Millicell ERS-2 (Millipore). Global gene regulation by AFTPH gene silencing in these cells was evaluated using microarray analysis (array type: U133 +2.0) and verified by RT-PCR. **Results:** In NCM460 cells during the growth arrest state, AFTPH gene-silencing downregulated ZO-1 protein expression (ICC and Western blot) in cell junctions, while CDH1 showed increased perinuclear localization (ICC). Knock down of AFTPH in NCM460 cells reduced TEER by 34.3%, and increased dextran (10 kMW) permeability ($p<0.05$), while lentiviral AFTPH overexpression reduced permeability ($p<0.05$). However, AFTPH gene-silencing showed no effect on permeability to dextran (3 kMW) in NCM460 cells. Microarray analysis showed that AFTPH gene silencing in NCM460 cells downregulated expression of genes involved in epithelial cell adhesion signaling (E-cadherin and β-catenin among others). The reduced expression E-cadherin and β-catenin after AFTPH gene silencing was verified by RT-PCR. **Conclusions:** Our results indicate that gene silencing of AFTPH in human colonocytes increased epithelial permeability to dextran (10 kMW), possibly by regulating expression and localization of genes related to epithelial cell adhesion signaling. These are the first results suggesting that AFTPH may be a new gene regulating intestinal epithelial permeability.

Acknowledgement: UCLA Vector Core and UCLA Clinical Microarray Core. Supported by NIH grant DK60729 (CP), P50 DK64539 (Project 2, CP), and CCFA Research Fellowship (IKML).

12.

Cathelicidin mimic Ceragenin CSA13 modulates *Clostridium difficile*-associated colitis in mice via a modification of intestinal microbiome.

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Background: *C. difficile* mediates intestinal inflammation by releasing toxin A (TxA), and antimicrobial peptide cathelicidin has been shown by our group to mediate anti-inflammatory effects against TxA. However, cathelicidin is a peptide that can be degraded easily, limiting its potential for clinical uses. A new non-peptide cathelicidin mimic called Ceragenin (CSA13) has been recently developed as an experimental drug. We hypothesize that CSA13 can be used to treat *C. difficile* infection. Here we investigated if CSA13 inhibits colitis and improves survival. **Materials and Methods:** CSA13 was provided by N8 Medical, Inc. (Primary infection study): c57BL/6 mice were infected with *C. difficile* (VPI10463 strain, 10(4) cfu). CSA13 (10 mg/kg) was administered subcutaneously every day (day 0-3). (Relapse study): The mice were infected with *C. difficile* (10(4) cfu) followed by oral vancomycin administration every day (day 0-2). Then the vancomycin administration was discontinued (day 3-5) and CSA13 was injected subcutaneously (50mg/kg, day 6 and 8). (TxA ileal loop study): TxA (10ug) was incubated with or without CSA13 (10mg/kg) in the ileal loop of mice for 4 hours. (*In vitro* study): Mouse Raw264.7 macrophages were exposed to CSA13 for 4 hours followed by incubation with TxA for 4 hours. **Results:** *C. difficile* infection caused 25% mortality and colonic damages with elevated histology scores, and increased colonic TNFalpha protein levels after 3 days. CSA13 prevented all mortality and significantly inhibited *C. difficile*-induced colonic damages, as shown by the reduced histology scores and colonic TNFalpha protein levels. Subcutaneous CSA13 administration increased the survival rate of *C. difficile* infected mice in the first 3 days, compared to the vehicle group (50% vs. 20% survival). Relapse occurred after withdrawal of vancomycin administration to *C. difficile* infected mice. The survival rate on day 10 was 33%, but the survival rate was increased to 47% in the CSA13 treated group. Body weight of vancomycin-mediated relapse group on day 7 was reduced to (81+/-2.2%) as it was partially reversed by subcutaneous CSA13 administration (89+/-1.9%, $p=0.0175$). 16S pyrosequencing shows that CSA13 treatment promoted the abundance of *Clostridiaceae* family of bacteria while reduced the abundance of *C. difficile* in the intestine. This family of bacteria competes with *C. difficile* and suppresses *C. difficile*-mediated intestinal inflammation. PCR array study shows that CSA13 (1uM) significantly reduced TxA-induced TNFalpha mRNA expression in mouse macrophages and ileal loops of mice, suggesting modest direct anti-inflammatory effect against toxin A. **Conclusions:** CSA13 can serve as a novel reagent to treat primary and relapses of *C. difficile* infection in mice via a specific modification of intestinal microbiome.

Funding: NIH K01 DK084256 AND R03 DK103964

13.

Antimicrobial peptide cathelicidin inhibits obesity in diabetic mice via inhibition of CD36 fat receptor expression

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Background: Obesity increases an individual's risk of developing diabetes, which is associated with debilitating complications. Despite medical advances, a new molecular target that can reflect the severity of obesity and diabetes in diagnosis or act as therapeutic agent is actively sought after. Cathelicidin is an antimicrobial peptide that possesses anti-inflammatory properties. Its expression is reduced in the skin of diabetic patients, but the overall metabolic role of cathelicidin in obesity and diabetes is not understood. We hypothesize that cathelicidin expression correlates to obesity and modulates fat mass and hepatic steatosis. **Materials and Methods:** (Human study) Human serum samples from non-diabetic and type II diabetic male and female patients of ages 18-70 were collected from UCLA. Cathelicidin levels in the serum samples were measured by ELISA. (Animal study) c57BL6 male mice were fed with a high fat diet for 8 weeks. After a streptozotocin injection to induce diabetes, the mice were fed with the high fat diet for 3 more weeks. Some groups were injected with cathelicidin and CD36 overexpressing lentiviruses. Fat and lean mass were measured by an EchoMRI machine. Liver steatosis was observed through Masson Trichrome staining. (In vitro study) Human primary mesenteric fat adipocytes and mouse 3T3-L1 differentiated adipocytes were used. **Results:** (Human study) Serum cathelicidin protein levels are significantly increased in obese, (BMI >30) non-diabetic patients, compared to non-diabetic patients with normal BMI values. Meanwhile serum cathelicidin protein levels are significantly lower in obese (BMI >30) type II diabetic patients than those of overweight (BMI 25-29.9) type II diabetic patients. (Animal study) Lentiviral overexpression of cathelicidin did not change the body weight but did reduce hepatic steatosis and decrease fat mass with corresponding increased lean mass of high fat diet-treated obese and streptozotocin-induced diabetic mice. Cathelicidin overexpression reduced mesenteric fat CD36 receptor mRNA expression and hepatic CD36 protein expression. Such changes were reversed by lentiviral CD36 overexpression. (*In vitro* study) Exposure of human mesenteric fat adipocytes, mouse 3T3-L1 adipocytes, and human HepG2 hepatocytes to cathelicidin significantly inhibited CD36 mRNA expression with reduced fat accumulation. Overexpression of CD36 reversed cathelicidin dependent inhibition of fat accumulation. **Summary:** Altering levels of circulating cathelicidin may indicate the development of obesity and diabetes. Cathelicidin inhibits the CD36 fat receptor and fat accumulation in adipocytes and hepatocytes, leading to reduction of fat mass and hepatic steatosis *in vivo*. Cathelicidin may be a novel therapeutic target against obesity in diabetic conditions.

Funding: NIH K01 DK084256 and R03 DK103964

14.

A High Protein Diet Reduces Body Fat Mass and Alters the Gut Microbiome, with Expansion of *Akkermansia,* in a Rat Model of Diet-Induced Obesity

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Background: We have previously demonstrated that a high protein diet (HPD) can reverse diet-induced obesity (DIO) in both animal models and in humans. A high fat diet (HFD) is well known to induce shifts in the composition of the gut microbiome in rodents and humans (Ussar S *et al.*, 2015; McAllan L *et al.*, 2014; Rosenbaum M *et al.*, 2015). However, it is unknown whether a HPD affects the microbiome induced by a HFD. **Aims:** To characterize the intestinal microbiome of rats with DIO switched to a HPD and to identify intestinal microbes that correlate with body fat mass. **Methods:** Male SD rats were fed a HFD (45% calories from fat - 5.5% from soybean oil and 39.4% from lard - and 17% from sucrose) for 12 weeks. Half of the rats were switched to a HPD (52% calories from protein, n=11) for 6 weeks and the other half remained on the HFD (n=10). Age-matched control rats were fed a normal diet (ND, 12% calories from fat and 29% from protein, n=8). Body composition was determined using rodent MRI (EchoMRI 700). 16S rRNA sequencing was performed on the luminal content of the cecum. Alpha and beta diversity analysis was performed at the level of 97% operational taxonomic units (OTUs). Multivariate analysis with DESeq2 was used to identify OTUs associated with diet and fat mass. **Results:** Rats on a HFD had 70% higher body fat mass than rats on a ND at 12 weeks. Switching to a HPD reduced fat by 29%

($p<0.01$) after 6 weeks compared to rats remaining on a HFD. The HFD and HPD groups had comparable microbial diversity, which was increased relative to rats on a ND as measured by the Shannon index, Chao1 richness, and phylogenetic diversity. Weighted UniFrac analysis demonstrated a shift in the intestinal microbiome composition of the HPD group compared to the HFD group ($p<10^{-5}$). The HPD group had increased abundance of 114 OTUs at a significance threshold of $q<0.05$, including *Akkermansia mucinophila*, *Fusobacterium*, segmented filamentous bacteria, *Ruminococcus* (21 OTUs), and *Bacteroides* (14). Only *Akkermansia mucinophila* ($q=0.008$) and an unclassified Clostridiales ($q=0.04$) had a statistically significant inverse correlation with fat mass after adjustment for diet. There was decreased abundance of 188 OTUs, including *Lactobacillus* (14), *Clostridium* (6), *Bifidobacterium* (4), *Roseburia* (2), and *Turicibacter* (2). Of these, an unclassified RF39 ($q=0.0001$) and a *Phascolarctobacterium* ($q=0.02$) were correlated with fat mass. **Conclusion**: Reduction of fat mass with a high protein diet is associated with extensive shifts in the intestinal microbiome. Abundance of *Akkermansia* was correlated with reduced fat, consistent with recent reports suggesting that *Akkermansia* protects against obesity (Everard A *et al.*, 2013; Schneeberger M *et al.*, 2015). These findings suggest that expansion of *Akkermansia* is a potential mechanism by which a high protein diet induces weight loss.

15.

Horizontal Gene Transfer of Accelerated Protein Evolution in the Human Gut Microbiome

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The human gastrointestinal (GI) tract houses approximately 10^{14} microbes of more than 1500 species, and represents one of the most diverse and complex microbial ecosystems in the biosphere. The mechanisms by which these microbes interact, exchange genetic information, and evolve new traits remain largely unknown. Diversity- generating retroelements (DGRs) are a novel family of genetic elements that diversify protein-encoding genes and accelerate protein evolution. Bioinformatic analysis discovered that DGRs are widely disseminated in the human gut microbiome, particularly within *Bacteroides* species. In this study, we have characterized the functionality and dissemination of a model retroelement *B. fragilis* 638R DGR, which encodes a diversified target protein, BdtA, with a lipoprotein signal sequence. Proteinase K sensitivity and immunostaining studies showed that BdtA is localized in the outer leaflet of the outer membrane and surface-exposed, supporting the hypothesis that *B. fragilis* has exploited DGRs to generate new ligand-binding specificities. Further examination of the 638R DGR locus and flanking regions suggested that it is carried on an integrative and conjugative element (ICE) subject to tight and complex regulation. *In vitro*, upon induction by BdiA (BF638R_2082), an AraC-like transcriptional regulator, the DGR-containing ICE was readily excised as detected by PCR assays. During colonization of gnotobiotic mice, ICE excision was also detectable in 638R cells even in the absence of BdiA. During *in vitro* mating, the ICE was horizontally transferred to *B. fragilis* 638R or *B. thetaiotaomicron* VPI5482 at a frequency of 10^{-6} to 10^{-7}, and next-generation sequencing (NGS) successfully detected DGR activity in 638R cells grown *in vitro* or during colonization of the mouse GI tract. Colon tissue-associated 638R cells demonstrated the highest DGR activity, followed by mucus-associated cells. Thus far, we have elucidated a molecular mechanism underlying the wide distribution of DGR- mediated protein evolution cassettes, which has the potential to increase bacterial fitness within the dynamic environment of the host gut.

16.

Loss of Corticotropin Releasing Factor (CRF) Immunoreactivity (IR) in the Colonic Enteric Neurons and Propulsive Motor Response to Lipopolysaccharide (LPS) in Vasoactive Intestinal Peptide (VIP) Deficient Mice

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Background: CRF is a key mediator of stress responses. Either central or peripheral administration of CRF can mimic stress-related alterations in the colon, including stimulation of defecation and diarrhea. We have established that CRF is located in the colonic enteric neurons and up-regulated by LPS and water avoidance stress. VIP, expressed in intrinsic non-adrenergic non-cholinergic enteric neurons, is involved in the regulation of gut motility

and immune homeostasis. We showed previously that intraperitoneal (IP) injection of VIP antagonist completely prevented fecal pellet output (FPO), diarrhea and Fos expression in the ileal submucosal plexus and the colonic myenteric plexus induced by IP CRF. **Aims:** To compare in VIP deficient ($VIP^{-/-}$) vs. wild type (WT) mice: 1. CRF expression in the colonic enteric neurons; 2. the response of colonic motility and inflammation to IP LPS. **Methods:** The proximal colon was collected from male $VIP^{-/-}$ and WT mice (6-8 weeks, n=4/group). Whole mount preparations of submucosal and myenteric plexus were prepared for CRF immunohistochemistry and double labeling of CRF and Hu C/D, a neuronal marker. The median eminence (ME) well known to exhibit strong CRF IR in the brain served as a positive control. In another set of experiment, $VIP^{-/-}$ and WT mice (4-5/group) received IP either LPS (200 µg/kg) or vehicle (saline). FPO was monitored every 30 min for 2 h, then the proximal colon was collected to detect mRNA levels of interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor (TNF)α and interferon gamma (IFNγ) by real time quantitative PCR. **Results:** In WT mice, CRF IR was detected in the ME and co-localized with Hu C/D in the submucosal and myenteric neurons. In $VIP^{-/-}$ mice, however, CRF IR completely faded away in the enteric plexus but still showed strong intensity in the ME. In IP saline groups, $VIP^{-/-}$ mice displayed a 1.6, 1.4, 1.5-fold decrease of FPO at 1, 1.5, 2 h compared to WT mice ($p<0.05$, $p<0.001$, $p<0.05$). LPS increased PFO by 1.9, 1.5 and 1.6 folds at 0.5, 1.5 and 2h after injection in WT mice compared to IP saline ($p<0.05$, $p<0.05$, $p<0.05$), while in $VIP^{-/-}$ mice, FPO values were not significantly different compared to IP saline. Basal mRNA levels of all cytokines detected in the colon were significantly higher in $VIP^{-/-}$ than WT mice. LPS increased significantly cytokine expressions in WT mice which were further enhanced in $VIP^{-/-}$ mice (Table 1). **Conclusions:** VIP gene deletion results in a reduction of basal and prevention of LPS stimulated propulsive colonic motor function that may involve the downregulation of the colonic CRF expression at the protein level. The enhanced LPS-induced inflammation in $VIP^{-/-}$ mice is consistent with VIP's anti-inflammatory action. Targeting VIP may be a potential strategy for a clinical treatment of stress-sensitive colonic motor disorders such as IBS and IBD.

Table 1. Effects of VIP gene deletion ($VIP^{-/-}$) on the mRNA expression of cytokines in the mouse proximal colon (Fold changes)

	WT/Saline	WT/LPS	$VIP^{-/-}$/Saline	$VIP^{-/-}$/LPS
	n=4	n=4	n=4	n=4
IL-1β	1.3±0.6	3.7±1.0*	4.7±0.8*	16.0±3.2# +
TNFα	1.1±0.2	2.4±0.6*	3.6±0.3*	3.5±0.2#
IL-6	1.1±0.3	1.8±0.4	2.5±0.3*	5.1±0.4#+
IL-10	1.0±0.2	2.8±0.5*	2.3± 0.3*	2.7±0.4
INFγ	1.2±0.3	4.2±1.1*	2.3±0.6	6.8±2.2

* $p<0.05$ vs WT/saline, # $p<0.05$ vs. WT/LPS, + $p<0.05$ vs. $VIP^{-/-}$/saline)

Abstracts of Posters
Clinical

17.

Mindfulness-Based Stress Reduction Improves Cerebral Blood Flow and Symptoms in Patients with Irritable Bowel Syndrome (IBS)

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Background: IBS is a disorder of brain-gut interactions, presenting as recurrent abdominal pain and altered bowel habits. Functional and structural brain alterations in sensory, affective and cortical control regions have been reported in IBS. Mind body treatments, such as hypnotherapy and mindfulness training, can alleviate IBS symptoms, but how these interventions impact the brain in IBS remains unknown. We hypothesized that successful treatment of IBS symptoms would be reflected by increases in cerebral blood flow (CBF) as measured with arterial spin labelling (ASL) in relevant brain regions. **Aim:** To assess changes in resting CBF in IBS patients before and after mindfulness based stress reduction (MBSR) training. **Methods:** The study included 41 Rome III positive IBS subjects (31 females and 10 males; mean age 32.3±8.9). All subjects underwent magnetic resonance imaging on a 3 Tesla Siemens scanner before and after a two-month, standardized course in MBSR. CBF was evaluated using ASL, an imaging technique that utilizes magnetically labeled water as an endogenous tracer to quantify regional blood flow, with levels of such flow reflective of neural activity. A custom pseudo-arterial spin labeling sequence with 40 unlabeled/labeled image pairs (3.4x3.4x6 mm voxel size, 24 slices, repetition time 7.2s/pair, echo time 12ms) was implemented. A questionnaire for IBS symptom severity (IBS-SSS) was administered at entry into the study and at the post MBSR MRI scan. Data was preprocessed with SPM software and the ASL Toolbox, and analyzed with the general linear model in SPM12. Pre- and post-meditation data were examined using SPM's "paired t-test" with covariate and "ANOVA" option to assess magnitude of change accounting for differing pre-intervention CBF levels. A-priori selected regions tested included the hippocampus, amygdala, cingulate cortex, posterior and anterior insula, and thalamus using small volume correction, and a whole brain analysis was also performed (family-wise error correction, FWE). **Results:** The mean change in symptom severity was a 67.8-point improvement (SD 104.4), with >49 being considered a clinically significant response. Responses did not differ by sex. CBF increased significantly post-MBSR in a cluster in the left fusiform gyrus at -30, -50, -10 (pFWE=0.002, T=6.27, Z=5.14), bilateral hippocampus (left p=0.015, T=4.61, Z=4.07; right p=0.022, T=3.84, Z=3.50), right posterior insula (p=0.012, T=3.96, Z=3.59), and bilateral thalamus (left p=0.044, T=3.71, Z=3.39; right p=0.042, T=3.52, Z=3.25). Regions showing no change were the anterior/mid cingulate, amygdala, and anterior insula. **Conclusion:** MBSR training leads to improved IBS symptoms as well as increases in cerebral blood flow in brain regions associated with sensation (thalamus, posterior insula), memory (hippocampus), and environmental recognition (fusiform gyrus).

18.

Effects of Parasympathetic Activation on Neural Responses during Emotional Reactivity and Regulation in Adolescents

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Parasympathetic tone is an important contributor to homeostatic balance and likely has significant influence on the flexibility of cortico-limbic networks involved in regulation of emotion and arousal. Adolescence is a neurodevelopmental period in which cortico-limbic circuitry has not yet reached maturation, likely resulting in greater difficulty with regulation of emotions. We sought to determine how a brief intervention for increasing parasympathetic tone via controlled breathing impacts adolescent emotion reactivity and regulation. Twenty-one adolescents (13-18 years old) underwent two fMRI scanning sessions in which resting state scans were acquired before and after they performed a reappraisal-based emotion regulation task, which involved proximal/distal

perspective taking while viewing images containing neutral or negatively arousing content (IAPS pictures). Prior to each scan session, participants engaged in either a controlled breathing exercise known to increase parasympathetic activation or a control condition in which they were guided through a relaxation exercise that involved normal breathing. Parasympathetic tone was measured using heart rate variability during rest in supine position before and after breathing or relaxation. Controlled breathing increased heart rate variability (an indication of greater parasympathetic tone), reduced fMRI activation to emotional images in occipital and posterior parietal areas, and increased activation in dorsolateral prefrontal and striatal regions. These preliminary analyses suggest that enhancement of parasympathetic activation results in greater fronto-striatal activation during emotional arousal. Given the role of fronto-striatal networks in self-regulation, such activation may reflect greater capacity for self-regulation resulting from increased parasympathetic tone.

19.

Comparing Connectomes Using Anatomical Connectivity within Extended Reward Network Regions Across Male and Female Obese Subjects

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Background: Alterations in key brain regions of an extended reward network have been linked to increased ingestive behaviors in obesity. Our recent publication demonstrated that a supervised learning algorithm can use regional connectivity with 90% accuracy to discriminate overweight from normal weight. These results allowed for the characterization of the effects of group (obese, overweight, lean) and sex on the anatomical connectivity within the extended reward network. Connectomes were used to visualize major disease and sex differences. **Methods:** White-matter was measured in 105 subjects. Scans were parcellated into 74 bilateral cortical and 7 subcortical structures and white-matter connectivity for each subject was estimated. GLMs were applied on connections within the extended reward network and customized contrasts were used to assess for disease and sex differences (FDR corrected significance $q< 0.05$). **Results:** 1. *Subject Characteristics:* There were 57 lean (22.06kg/m^2, 34 females), 32 overweight (26.30kg/m^2, 11 females), and 16 obese (33.24kg/m^2, 8 females) individuals. 2. *Obese vs. Lean:* Compared to females, smaller differences in connectivity were found between obese and lean subjects in males in reward-reward regions (e.g. brainstem to NAcc ($\beta = -1.127$, $q= 0.031$), CaN to OFG ($\beta = -0.289$, $q= 0.030$)). Compared to females, smaller differences in connectivity were found between obese and lean subjects in males in reward to emotional arousal regions (e.g. Amyg to OFG ($\beta = -14.603$, $q< 0.001$), to NAcc ($\beta = -0.143$, $q= 0.016$)). In obese males, ETI emotional ($F= 4.62$, $p= 0.03$), ETI sexual ($F= 6.32$, $p= 0.01$), and ETI total ($F= 6.92$, $p= 0.01$) scores were larger compared to lean males. Significant correlations were found between ETI sexual, ETI total, depression, and VSI scores in many of the reward-reward connections in obese compared to lean individuals. 3) *Obese vs. overweight:* Compared to females, smaller differences in connectivity were found between obese and overweight subjects in males in reward-reward regions (CaN to OFG ($\beta = -1.664$, $q= 0.025$)), but greater differences in connectivity were found from NAcc to OFG ($\beta = 0.614$, $q= 0.041$)). Compared to females, smaller differences in connectivity were found between obese and overweight subjects in males in reward to emotional arousal regions (e.g. Amyg to OFG ($\beta = -14.173$, $q= 0.002$), to NAcc ($\beta = -0.143$, $q= 0.030$)). In males, there were significant differences in anxiety ($F= 4.06$, $p= 0.04$) and depression ($F= 5.93$, $p= 0.2$) scores compared to females. In overweight compared to lean individuals, significant correlations were found between CSQ and PSS scores and reward-reward connections. **Discussion:** The connectomes demonstrate that the anatomical networks of regions within the extended reward network vary by both BMI and sex. Specifically being obese and female is associated with more local and regional connectivity between regions associated with increased dopamine production, and less information propagation was observed in the cognitive frontal regions.

Funding: Supported by NIH grants: P30 DK041301, R01 DK048351, R01 HD076756, P50DK64539, Pilot scans were provided by the Ahmanson-Lovelace Brain Mapping Center, UCLA

20.
Functional Network Properties of Brain Regions in Irritable Bowel Syndrome

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Introduction: Irritable bowel syndrome (IBS) may be associated with the reorganization of brain networks (Mayer et al 2015) leading to the emergence of different communication pathways between brain regions. Graph theory conceptualizes brain regions as nodes and the connections emanating from them as edges. Nodes that are central to the flow of information within and between brain networks are known as hubs. Previous studies have characterized the distinctive disease-related changes in these hubs for various brain disorders (Crossley et al 2014). **Aims:** To demonstrate: (1) differences in the centrality of the brain regions involved in the salience, emotional arousal, sensorimotor, and central executive network when comparing IBS subjects to healthy controls (HCs) and (2) that these changes are moderated by sex. **Methods**: Structural and resting-state functional images were obtained in 275 subjects (61 male HCs, 63 female HCs, 45 male IBS, 106 female IBS). The Destrieux and Harvard-Oxford brain atlases were used to perform image segmentation and regional parcellation to divide the brain into 165 regions. The CONN toolbox was used to construct subject-specific functional networks based on existing functional connectivity. Network metrics related to centrality were used to further characterize hub regions: Strength, Betweenness Centrality, Characteristic Path Length, and Clustering Coefficient. Group differences in hubs were tested using a regression. Correlations were conducted between significant hub regions and clinical variables. **Results**: *Functional hubs*. *1) Cortical inhibition*. Frontal regions involved in cortical inhibition, including the bilateral middle frontal gyrus, triangular part of the inferior frontal, and the orbital gyrus, were identified as hubs in HCs but not IBS. *2) Sensory regions.* The posterior insula, as well as various somatosensory regions, (bilateral subcentral gyrus, precentral gyrus, postcentral gyrus) were identified as hubs in IBS but not in HCs. *3) Emotional arousal.* The pregenual anterior cingulate was identified as a hub only in female IBS subjects. *Symptom correlations*. Centrality of the orbital gyrus in male IBS subjects showed moderate negative correlations with symptom severity (r=-.33, p=.04), state anxiety(r=-.50, p=.01), and catastrophizing (r=-.49, p=.04). In contrast, centrality of the orbital gyrus showed small positive correlations with bloating and symptom severity (r's=.21, p=.04). **Conclusions**: These findings support the idea that the functional architecture of the sensorimotor, cortical inhibitory, and emotional arousal regions differs in IBS compared to HCs. Differences are moderated by sex and are shown to correlate with some self-reports of symptom severity.

21.
Morphological Brain Alterations in Localized Provoked Vulvodynia (LPVD)

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Background: Localized Provoked Vulvodynia (LPVD) affects approximately 16% of females. While the biological mechanisms underlying the symptoms are poorly understood, both peripheral and central abnormalities, including alterations in sensory processing, have been reported in LPVD. **Aims:** To determine: 1) Alterations in morphological brain regions in LPVD compared to healthy controls (HCs) 2) to irritable bowel syndrome (IBS) and 3) Correlations between morphological differences and clinical variables. **Methods:** Structural MRI was obtained in 119 age-matched premenopausal females (45 LPVD, 45 HC, 29 IBS). Image segmentation and regional parcellation were performed dividing the brain into 165 regions. Volume (V), cortical thickness (CT), mean curvature (MC), and surface area (SA) were calculated. General linear modeling was used to test for differences between the groups. Correlations were conducted between significant structural differences and clinical variables. **Results**: **1)** LPVD vs HC: LPVD demonstrated higher left caudate nucleus V (p=.05), right medial orbital sulcus MC (mOS, p=.02), left aMCC CT (p=.05), left postcentral sulcus CT (PostCS, p=.01) and right middle frontal gyrus CT (MFG, p=.04). LPVD showed smaller left precentral gyrus SA (PreCG, p=.01) and MC of various somatosensory regions. **2)** LPVD vs IBS: LPVD had greater volumes in left paracentral lobule (p=.03), right parahippocampus (ParaHipp, p=.02), left superior frontal sulcus (SFS, p=.02), and left SFG (p=.01), but decreases in right mOS V (p=.01), left mINS MC (p=.04), left PreCG MC (p=.03), but greater right aMCC MC

(p=.03) and right OG (p=.01). LPVD had greater CT in various somatosensory regions. **3)** Correlations: Compared to HCs, left PreCG SA showed negative correlations with overall pain catastrophizing (r=-.42) and helplessness (r=-.48) in LPVD. Compared to IBS, there were various significant negative correlations in LPVD: right mOS V with pain helplessness (r=-.38), left SFG V with pain magnification (r=-.43) and muscle tenderness (r=-.43), left PreCG SA with various vaginal muscle tenderness scores (r~-.40), pain catastrophizing scores (r~-.5) and right PosCS MC with pain rumination (r=-.37). There were also various significant positive correlations: left paracentral lobule V with pain intensity (r=.40), right ParaHipp V with vulvar pain at 5 o'clock (r=.39) and at 6 o'clock (r=.53), left pINS SA with pain rumination (r=.42), pain duration (r=.55), and right OG MC with vaginal muscle tenderness at 6 o'clock (r=.38). **Conclusions:** These structural findings in LPVD are consistent with previously reported morphological alterations in pain modulation and sensorimotor regions found in chronic pain conditions. The greater disease-related morphological differences and the correlations with several clinical measures in LPVD were associated with those regions often related to sensorimotor and tonic contractions of pelvic floor muscles.

Funding: Supported by NIH grants: P30 DK041301, R01 DK048351, R01 HD076756, P50DK64539, Pilot scans were provided by the Ahmanson-Lovelace Brain Mapping Center, UCLA

22.
Changes in Microbiome Related to Daily Pomegranate Consumption

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Our previous study has shown that daily consumption of pomegranate juice has been associated with improved cognitive and other memory functions in humans associated with an increase in plasma pomegranate metabolites. The health benefits of pomegranate (POM) consumption are attributed to ellagitannins. Due to their molecular size ellagitannins are not absorbed in the small intestine but metabolized in the intestine by the microbiota to ellagic acid and urolithins. The objective of the present study was to determine changes in the gut microbiota in individuals consuming pomegranate extract. Twenty healthy participants consumed 1000 mg of POM extract daily for four weeks. Based on urinary and fecal content of the POM metabolite urolithin A (UA), we observed three distinct groups: 1) individuals with no baseline UA presence but induction of UA formation by POM extract consumption (n=9); 2) baseline UA formation which was enhanced by POM extract consumption (N=5) and 3) no baseline UA production, which was not inducible (N=6). Compared to baseline the phylum *Actinobacteria* was increased and *Firmicutes* decreased significantly in individuals forming UA (producers). *Verrucomicrobia (Akkermansia muciniphila*) was 33 and 47-fold higher in stool samples of UA producers compared to non-producers at baseline and after 4 weeks, respectively. In UA producers, the genera *Butyrivibrio, Enterobacter, Escherichia, Lactobacillus, Prevotella, Serratia* and *Veillonella* were increased and *Collinsella* decreased significantly at week 4 compared to baseline. The consumption of pomegranate resulted in the formation of its metabolites in some but not all participants. POM extract consumption may induce health benefits secondary to changes in the microbiota.

23.
Translational Neuroscience of Caregiver Stress and Depression and response to the Meditation: Mind-Body Connection

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We recruited 49 depressed family dementia caregivers (mean age 60.3 yrs (SD=10.2)), 39 were randomized and completed either KK meditation or listening to relaxation music for 12 minutes per day for 8 weeks. Severity of depressive and anxiety symptoms, burden, and coping were assessed at baseline and over the course of the study. The mean HAMD score at baseline was 11.6 (SD=4.1). Telomerase activity was examined in the monocytes before and after the study. Groups differed on the health functioning outcomes (i.e., SF-36 role emotional and energy scale scores), global cognitive measure (MMSE) and measures of attention and executive

function (Trails A/B) (p<.05), which was associated with decreased FDG-PET brain metabolism in right inferior frontal lobe. Improvement in coping and cognition correlated with increased telomerase activity in the meditation group (43%) compared the relaxation group (8%), which was significant after controlling for age and the duration of stress **(p=0.03)**. We also performed genome-wide transcriptional profiling in PBMC samples (microarrays) and observed the pattern of increased NFkappaB-related transcription of pro-inflammatory cytokines and decreased IRF-related transcription of innate antiviral response genes, which was also supported by the assays of peripheral NF-kappa-B expression. **fMRI showed** different patterns of activation distinguishing KK meditators from controls, meditators showed higher activity in a functional network including the anterior cingulate, fronto-orbital cortex and insula, regions that are relevant to mood and cognitive regulation. These findings suggest that brief meditation in mildly depressed individuals can lead to improved coping and cognition compared to relaxation groups. In addition, these findings are accompanied by increases in the levels of telomerase, downregulation of inflammatory signaling pathways and cellular aging, and the "brain fitness" effect of meditation in the frontal lobes on fMRI.

24.

Children with Functional Gastrointestinal Disorders Display Structural Brain Alterations Compared to Healthy Control Subjects

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Background: Functional gastrointestinal disorders (FGIDs) are seen commonly in both adults and children. However, far fewer studies have investigated the role of the brain gut axis in pediatric FGIDs. It is known that structural changes exist in the brains of adult patients with irritable bowel syndrome (IBS). These changes include increased prominence of the somatosensory cortex, with reduced insula and cingulate cortex gray matter. **Aim:** To determine if structural brain changes seen in adult IBS can already be identified in children with IBS symptoms. **Methods:** Female children aged 7 to 17 with IBS and/or FAP were enrolled with inclusion of aged matched healthy controls. Neuroimaging was performed at two sites, Baylor College and University of California Los Angeles, using standardized protocols. Both sites employed a 3 Tesla Siemens scanner with a 12 channel head coil and acquired a high-resolution T1-weighted magnetization-prepared rapid acquisition gradient echo scan over 10 minutes with the following parameters: TE 2.98ms, TR 2300ms, FOV 256, Flip angle 9 degrees, duration 5:12. Subjects with greater than 2 mm movement in any direction or poor segmentation were excluded. Brain segmentation and regional parcellation was performed using FreeSurfer software utilizing the Destrieux and Harvard-Oxford atlases and gray matter volume was determined. A priori regions of interest were selected to include sensorimotor (pre and post central gyri/sulci), salience (anterior insula subregions) and emotion related regions (amygdala, anterior and anterior mid cingulate cortices). Statistical comparison between FGID and healthy was implemented in a general linear model accounting for total gray matter volume, site, and age. **Results:** 56 subjects had adequate neuroimaging data to include in the analysis. The mean age of the FGID and healthy groups were not statistically different: FGIDs 10.46 years, SD 2.6; Healthy 11.2 years, SD 2.9; p=3.2. Two regions showed decreased gray matter volumes in the FGID group: the left amygdala (beta=57.2, p=.027) and the circular sulcus of the anterior insula (beta= -33.5, p=.042). One region showed an increase in gray matter volume in the FGID group, the superior precentral sulcus (beta=128.3, p=.029). **Conclusions:** Similar to adults with IBS, children with FGIDs show decreased gray matter in regions associated with salience processing (anterior insula) and emotion regulation (amygdala). Children with FGIDs show increased gray matter volume in a sensorimotor region (precentral gyrus), a region that has been reported to have functional changes in adults with chronic pain. These findings suggest that the brain changes identified in FGIDs as adults are not merely a consequence of long standing symptoms, but may be a predisposing factor for altered sensory perception already present in young children.

25.

Corticotropin-Releasing Hormone Receptor 1 (CRH-R1) Polymorphisms are Associated with Irritable Bowel Syndrome (IBS) and Acoustic Startle Response (ASR)

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Background: Corticotropin-releasing hormone receptor 1 (CRH-R1) in the amygdala plays an important role in the activation of central stress circuits. Genetic factors may contribute to the hyperresponsiveness of these circuits, which has been implicated in the pathophysiology of irritable bowel syndrome (IBS). It is not known if single nucleotide polymorphisms (SNPs) of the CRH-R1 are associated with altered amygdala function, as indexed by enhanced symptom related anxiety and by the enhanced acoustic startle response (ASR) found in IBS. **Aims:** To determine if CRH-R1 SNPs are associated with: 1) a diagnosis of IBS, 2) gastrointestinal (GI) symptoms, and 3) ASR. **Methods:** Salivary samples for DNA and symptom questionnaires were obtained in IBS patients and healthy controls (HCs). 3 CRH-R1 SNPS were genotyped. ASR (EMG activity of orbicularis oculi) were obtained during safe (no chance of shock), anticipation (Anticip; shock may occur) and threat (shock likely) conditions in a subset of patients to assess stress response to a visceral abdominal threat. The optimal genetic model was used for testing associations of SNPs with IBS status and ASR. Regression analyses were used to evaluate associations of each SNP with IBS status, clinical traits and ASR measures after controlling for sex, race, current anxiety and depression symptom (HAD) scores. P value <0.05 was considered significant. **Results:** 235 IBS patients (mean age 37.5 yrs, 74% F) and 264 HCs (mean age 32.1 yrs, 70% F) were studied. Of these subjects, 57 IBS and 41 HCs underwent the ASR protocol. 1) *IBS diagnosis:* The presence of IBS was associated with the major allele for all three CRH-R1 SNPs (rs110402 [p=0.015], rs242924 [p=0.025], and rs7209436 [p=0.009]). 2) *Symptoms:* Among IBS patients, the major allele for all three SNPs (rs110402 [p=0.035], rs242924 [p=0.017], and rs7209436 [p=0.065]) was associated with increased visceral sensitivity index (VSI) scores, a measure of GI symptom anxiety, but not overall IBS or abdominal pain severity. 3) *ASR*: Within subjects who have at least one copy of the major allele for rs110402 or rs242924, IBS had significantly lower ASR compared to HCs during the threat conditions (see Figure, p=0.002 for both). In contrast, IBS patients with homozygous minor alleles for CRH SNPs had a numerically higher ASR to threat compared to HCs. In addition, within the IBS group, CRH-R1 SNPs were associated with a graded increase in ASR to threat CRH-R1 SNPs. Patients with at least one copy of the major allele for rs110402 or rs242924 had significantly lower ASR compared to patients with no copies (p=0.008 for both). **Conclusion:** CRH-R1 genotypes were associated with IBS status, GI symptom anxiety and ASR to threat. These findings support that CRH-R1 contributes to the dysregulated stress responsiveness in IBS.

26.

Effect of Bariatric Surgery on Regional Topology of Functional Networks

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Background: Obesity is a growing health care concern around the world with an estimated 700 million obese individuals in 2015. Bariatric surgery has become a popular clinical procedure for sustained weight loss, yet despite its extensive application, the mediators of weight loss after surgery are widely unidentified. Alterations in key brain regions of the extended reward network have been linked to increased ingestive behaviors in obesity. Graph theory allows us to conceptualize brain regions as nodes and the connections as edges. The topology of large-scale anatomical brain networks can be described by quantifying local measures of centrality, such as *strength* and *node betweeness,* that index a brain region's contribution to the network's structural integrity and information flow. We hypothesized that differences in regional topology of the extended reward network would be observed post-surgery. **Aims**: The aims of this study were to determine: 1) Improvements in food addiction

behaviors and clinical variables one month post-surgery 2) Changes in topology of functional networks post-surgery. 3) Correlations between changes in network metrics post-surgery with measures of obesity pre-surgery. **Methods**: Resting-state functional images were obtained in 7 obese female subjects. MRI scans were obtained pre and post-prandial at baseline and then one month post-bariatric surgery. Based on graph theory we computed and compared network measures of centrality for brain regions using linear contrast analysis, the GTG toolbox, and in house Matlab code. Significance was determined via non-parametric permutation tests. **Results**: 1) Post surgery subjects showed the following decreases: Weight (13.6 kg), BMI (4.7kg/m^2), Waist (8.24cm), HAD Depression score (66%), HAD Anxiety Score (49%), Yale Food Addiction Score symptom count (30.7%). 2) Centrality of reward regions (left (L) hippocampus, L nucleus accumbens, amygdala, right (R) putamen), R subcallosal gyrus, L middle frontal gyrus, anterior insula, and sensory regions (bilateral posterior insula, bilateral central sulci, bilateral anterior midcingulate cortex, R superior part of the precentral sulci (SupPrCs)) were all reduced post surgery (p's=.0001-.04, hedges'g= 1.71 to 3.46). 3) Several of these regional-network-metrics shown to change with surgery were strongly correlated with measures of obesity pre-surgery. For example increased betweenness centrality of the R SupPrCs was associated with excess weight (r=.80, p=.03), BMI (r= .77, p=.04), Fat mass (r=763, p=0.046). **Conclusion**: Measures of centrality were reduced post-surgery in dopamine rich regions associated with greater impulsivity and food intake. There were also significant improvements in weight related measures and food addiction behaviors. It is possible that decreases in information flow of reward regions post-surgery may be associated with decreased sensitivity and thus decreased overconsumption of calorie dense foods.

Funding: Supported by NIH grants: P30 DK041301, R01 DK048351, R01 P50DK64539, Pilot scans were provided by the Ahmanson-Lovelace Brain Mapping Center, UCLA

27.

Bariatric Surgery Is Associated with Changes in the Brain's Reward System Architecture and Eating Behaviors

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Background: Obese subjects display alterations in brain's reward system and eating behaviors favoring highly palatable food. Bariatric surgery results in sustained weight loss, however, the underlying mechanisms are incompletely understood. Bariatric surgery may elicit changes in the brain's reward system leading to adjustments in hedonic eating behaviors. **Aims**: We aimed to test 2 hypotheses: 1) Laparoscopic sleeve gastrectomy (LSG) results in changes in appetite and hedonic eating behaviors 2) These changes are associated with morphometric changes in brain's gray matter (GM). **Methods**: 8 adult obese women (age:39.5±8.7yrs) underwent LSG and had their BMI, fat mass, hedonic eating scores (Yale Food addiction scale and desire to eat high vs. low calorie foods), hunger/satiety scores and brain MRIs obtained at 1-month pre- and post-surgery. Freesurfer analysis yielded gray matter (GM) volume measured bilaterally in regions of interest within the reward system. Paired test was used to test for changes in variables after surgery. Associations between obesity measures, eating behaviors and GM metrics were explored using Pearson correlations. **Results**: Surgery resulted in 1) a decrease in BMI from 43.4±6 to 38.8±6.2 (hedges [Cohen's' effect size d adjusted for small samples=.75 p<0.0001) 2) A decrease in hunger scores (72.5±10.3 vs 41.2±24.7, pre vs post, g=1.65,p=0.003) and an increase in postprandial satiety levels (29.5±19.4 vs 82.8±19.7, pre vs post, g = -2.73, p=0.001). 3) A reduced desire to eat sweets (7.7 vs. 3.6, pre- vs. post-op, p<0.05), and a reduction in food addiction scores (4.29±2.3 vs 1.1±0.4, pre vs post-op, p=0.015). Lower total gray matter volume was significantly associated with feeling hungrier at fast, both before (r=-.694, p=0.056) and after surgery(r=-.735 p=0.038); however, GM volume did not correlate with obesity measures. GM volume of putamen, nucleus accumbens, amygdala and posterior insula was associated with measures of adiposity prior to surgery and they were altered after surgery. Greater volume at the left putamen was associated with excess weight (r=.80, p=0.06) and BMI (r=.85, p=0.03). After surgery, this region's volume was significantly reduced (t=2.22, p=.062, Hedges g=.84). Moderate effect size changes in volume post surgery were observed for right posterior insula (t=2.55, p=.038, g=.67) and left amygdala (t=1.85,p=.11, g=.40). **Conclusions**: LSG results in reduced in measures of obesity, appetite and hedonic eating, as well as in structural changes in several of the

brain's reward system regions. These findings suggest an important role of surgery related brain changes in the effectiveness of LSG weight loss. Future studies in larger samples are required to confirm these observations.

28.

Advanced Gut Microbial Composition Analysis Is Able to Discriminate between Before and After Laparoscopic Sleeve Gastrectomy Status

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Background: Alterations in gut microbiome have been associated with obesity. Weight loss surgery results in significant changes in the anatomy, function and intraluminal environment of the gastrointestinal tract and therefore affects the gut microbiome. Limited data is available on the effect of laparoscopic sleeve gastrectomy (LSG) on gut microbiome and on surgically induced weight loss**. Hypothesis:** 1) LSG results in changes in gut microbiota composition 2) Alterations in gut microbiome may have an independent effect on surgically-induced weight loss. **Methods:** Six obese women (age:42.8±7.3yrs) underwent LSG. Their weight, BMI, calorie intake (2-day food dairy), and stool samples for microbiota composition were obtained at 1-month pre- and post-surgery. Paired Ttest was used to test for changes in variables after surgery. 16S ribosomal RNA gene sequencing was performed on stool samples. Multilevel sparse partial least squares discriminat analysis was applied to genus level abundance data to determine whether microbial composition could discriminate the sample into pre and post-surgery status. **Results:** Subject's weight decreased from 115.1±27.2 (Mean(SD) before, to 103±25.4 kg (p=0.0002) after surgery. BMI also decreased from 43.6±6.4 to 39.2±6.9 kg/m^2 (p<0.0001) after surgery. Sleeve gastrectomy resulted in a significant decrease in daily calorie consumption (1844.5±628.9 vs. 614.2± 403 calories/day pre vs post-surgery, p=0.03), with a significant decrease in calories from carbohydrates (870.4±287.5 vs 235.7±185,3 calories/day pre vs. post-surgery, p=0.02). Microbial signatures differentiated pre vs post-surgery status (Figure 1A). Microbial signature 1 was responsible for 90% of discrimination between pre vs post-surgery status and was comprised of 5 bacteria including Atopobium, a genus of Actinobacteria; Bacteroides , member of Bacteroidetes; and Bulleidia, member of Firmicutes as main contributors. Hierarchical clustering (Figure 1B) indicated that pre-surgery samples and post-surgery samples cluster together without mixing of samples between different states. **Conclusions:** Laparoscopic Sleeve Gastrectomy results in a reduction in obesity measures, daily calorie intake and profound changes fecal microbial composition. Microbial composition discriminated pre and post-weight loss surgery status. Ongoing recruitment in this study will help us to find if changes in microbial signatures after bariatric surgery are associated with weight loss beyond the surgery effect on diet and calorie intake.

Figure 1A

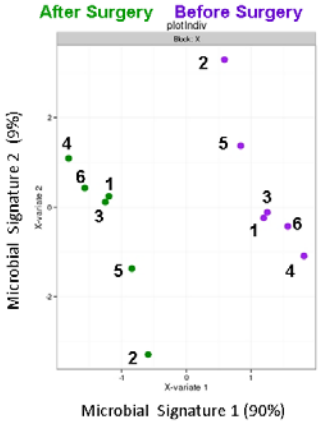

Figure 1B

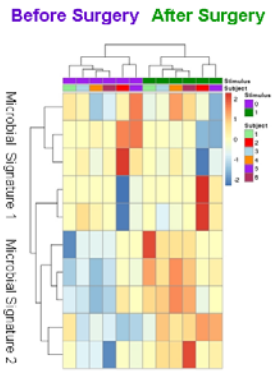

Figure 1A. Each dot is a sample, purple color for before surgery, green color for after surgery. Numbers represent each subject. Figure 1B shows genu composition for each microbial signature that was used in Figure 1A to discriminate between pre (purple) and post-surgery (green) status.

29.

Mindfulness Based Stress Reduction improves Irritable Bowel Syndrome (IBS) symptoms via specific aspects of mindfulness

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Introduction: Mindfulness Based Stress Reduction (MBSR) has been shown to improve IBS in controlled trials[1,2]. Being mindful is characterized as heightened attention to present moment experiences with an open, nonreactive, non-judging attitude. MBSR is the most widely studied clinical mindfulness program for IBS and other medical disorders, however it is not clear from prior studies which aspects of mindfulness are most relevant to IBS and could be targeted in future programs to improve treatment outcomes. **Aims:** To examine IBS clinical outcomes from MBSR and define which facets of mindfulness change are most associated with improvement in IBS symptoms, IBS specific quality of life (QOL) and GI symptom-related anxiety. **Methods:** 61 ROME III+ IBS patients (mean age=34.1±11.5 yrs, 47 F) were studied before and at the conclusion of an 8 week, 20 hour, MBSR training program. Measures included: IBS severity (IBS Severity Scale, IBS-SSS), IBS specific QOL (IBS-QOL), GI symptom-related anxiety (Visceral Sensitivity Index, VSI), and the Five Facet Mindfulness Scale (FFMS) The FFMS measures distinct aspects of mindfulness including; Observing (Attention to present moment, Observe), Describing (labeling internal sensations, Describe), Acting with awareness (Actaware), Nonjudging of inner experience (NonJudge) and Nonreactivity to inner experience (NonReact). Pre-post treatment difference scores were computed for each variable and multiple linear regression was used to examine FFMS subscales for the strongest predictors of IBS outcome changes. **Results:** IBS symptom responder rate was 71% (>49 point change on IBS-SSS) and there was a significant pre-to-post treatment change for all the FFMS subscales except Describe (see Table 1). Regression indicated FFMS change was significantly predictive of IBS-SSS change (R= .49, p=.009) and a stepwise regression indicated that change in the ActAware (p=.02) and Describe (p=.02) subscales were the best predictors of IBS-SSS improvement. In separate regressions ActAware change also had the strongest independent association with improvement in VSI (p=.02) and IBSQOL (p=.002). **Conclusions:** MBSR training has a large positive impact on IBS symptoms, symptom-related anxiety, and QOL. Although changes in 4 of the 5 measured facets of mindfulness are found in MBSR-trained IBS patients, the results suggest that increases in the subscales representing labeling internal sensations (Describe) and acting with awareness (Actaware) may be particularly important for IBS patients. This knowledge can be used to refine the standardized MBSR protocols specifically for IBS, which may assist in achieving improved outcomes and/or decreased time burden to patients and providers.

Table 1: Outcome variables

Measure	Pre-MBSR	SD	Post-MBSR	SD	p value	D' Effect Size
IBS-SSS	268.93	76.30	176.90	98.59	<.001	.95
FFMS Observe	26.31	5.22	29.15	5.38	<.001	.54
FFMS Describe	28.45	6.02	29.63	6.54	.066	.25
FFMS ActAware	24.97	5.99	27.15	5.75	.002	.37
FFMS Nonjudge	26.50	8.40	30.19	7.44	<.001	.67
FFMS Nonreact	22.03	5.14	24.02	5.23	.002	.41
VSI	44.94	16.00	30.00	18.14	<.001	.99
IBSQOL	58.41	21.77	71.72	19.90	<.001	.80
HAD Anxiety	8.26	4.10	6.59	4.13	<.001	.49
HAD Depression	3.68	2.87	2.84	2.78	.022	.32

References: [1]Gaylord SA, et al., American J Gastro.2011;106(9):1678. [2]Kearney DJ et al., Aliment Pharm Ther 2001;34(3):363.

30.
Differential Colonic Mucosal mRNA Expression in IBS with Constipation

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Background: Studies evaluating mucosal mRNA expression associated with irritable bowel syndrome with constipation (IBS-C) are few and sample sizes are small. **Aims:** To evaluate mucosal mRNA expression in IBS-C patients in comparison to healthy controls (HCs) and confirm differentially expressed genes in our sample to publically available microarray data. **Methods:** 10 Rome III + IBS-C patients and 10 age and sex matched HCs underwent sigmoidoscopy with sigmoid colon biopsies, from which total RNA was extracted. Gene expression (mRNA) was measured using ArrayStar (Agilent platform) microarrays. Differential expression was identified using the limma package in R. A Benjamini-Hochberg-adjusted p-value <0.05 was considered significant. Two publically available microarray (Affymetrix) analyses of sigmoid biopsies collected by Aerssons et al (E-TABM-176, 32 IBS-C and 55 HCs) and rectal biopsies from Spiller et al (GSE36701, 18 IBS-C and 21 HCs) were accessed and analyzed with the affy package for R and differential expression was determined using limma. An unadjusted p-value threshold of <0.05 was used for significance threshold for matching genes in the external data-sets. **Results:** In our dataset, 1149 unique mRNAs were differentially expressed between IBS-C and HCs with an FDR of <0.05. There were 17 mRNA that were differentially expressed (in the same direction) in our sample as well as in both external datasets (13 upregulated and 4 downregulated in IBS-C vs. HCs). A heatmap of these mRNAs is shown in Figure 1. **Conclusions:** Analysis of publically available gene expression data is useful has identified mRNAs that are are differentially expressed in IBS-C in multiple different samples. Several of these genes have potentially relevant functions. PVRL1, also known as nectin-1, is a protein in the adherens junction of epithelial cells. It was shown to be downregulated by miR-199a-5p which has been associated with increased permeability in the bladder and gut. In the central nervous system, nectin-1 has been implicated in early life stress induced impairment in hippocampal synaptic plasticity. PCDHA3 is also a cell-adhesion molecule. ST3GAL3 is involved in pre-NOTCH signaling. Deficiency in mice results in enhanced allergic response. Finally, TXLNG is upregulated by lipopolysaccharide. These genes are among those that will be further evaluated with targeted replication.

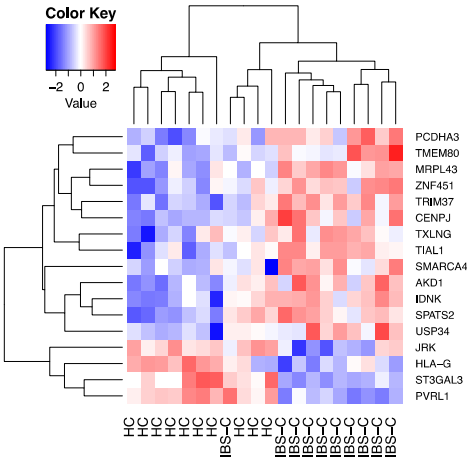

Figure 1: Heatmap of hierarchically clustered mRNAs differentially expressed in our dataset which were replicated in microarray data from two publically available well-phenotyped populations. Red indicates up-regulation and blue indicates down-regulation. IBS-C, irritable bowel syndrome with constipation, HC, healthy control; TMEM80, transmembrane protein 80 isoform 1; PVRL1 (poliovirus receptor-related protein 1 isoform 2 precursor; ST3GAL3, CMP-N-acetylneuraminate-beta-1,4-galactoside alpha-2,3-sialyltransferase isoform h; TRIM37, E3 ubiquitin-protein ligase TRIM37; AKD1, adenylate kinase domain-containing protein 1 isoform 2; SPATS2, spermatogenesis-associated serine-rich protein 2; ZNF451, zinc finger protein 451 isoform 2; JRK, jerky protein homolog isoform a; IDNK, idnK, gluconokinase homolog (E. coli); PCDHA3, protocadherin alpha 3; CENPJ, centromere protein J; MRPL43, 39S ribosomal protein L43, mitochondrial isoform a; SMARCA4, transcription activator BRG1 isoform D; TXLNG, gamma-taxilin isoform 2; HLA-G, major histocompatibility complex, class I, G; USP34, ubiquitin carboxyl-terminal hydrolase 34; TIAL1, nucleolysin TIAR isoform 1.

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Disease-Related Microstructural Differences in the Brain in Females with Localized Provoked Vulvodynia

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Background: Localized provoked vulvodynia (LPVD) affects almost 15% of females and is characterized by localized sensitivity of the vulvar vestibule. Very little research has been performed characterizing central abnormalities in LPVD using neuroimaging. The few studies that are available indicate that neural alterations may play a causal role in symptom generation or are secondary responses to the chronic pain condition. **Main Objective:** The aim was to utilize diffusion tensor imaging (DTI) to identify unique microstructural differences in the brain. We compare LPVD to healthy controls (HC) to assess whether alterations in fractional anisotropy (FA) and mean diffusivity (MD), which are measures of cohesive axonal orientation and tissue compactness, are specific to pain, and with IBS to determine distinct or shared mechanisms with another chronic pelvic-pain disorder. **Methods:** DTI MRIs were conducted in a sample of 87 age-matched premenopausal females (29 HC, 29 LPVD, 29 IBS). FA and MD were processed using the FSL Diffusion Toolbox. Voxelwise and atlas-based (ICBM White Matter and Harvard-Oxford Subcortical) region of interest analyses were performed using pairwise t-tests with age and BMI as covariates. **Results:** LPVD generally presented both increased FA and increased MD when compared to both HC and IBS, with the differences with HC being more widespread. FA and MD differences were in general not co-localized to the same region, and FA differences tended to occur in the right hemisphere while MD differences tended to occur in the left hemisphere. *LPVD vs. HC*: Patients with LPVD demonstrated significantly higher FA in white matter regions connecting subcortical or spinal cord regions to cortex (internal and external capsules, corona radiata, cingulum bundle), white matter regions connecting sensory cortex with motor or cognitive cortex (sagittal stratum, superior longitudinal fasciculus), and in subcortical regions (putamen and amygdala). Higher MD was present in subcortical regions in the left hemisphere (thalamus, amygdala, putamen, caudate, and pallidum), as well as multiple white matter regions. *LPVD vs. IBS*: FA differences were less extensive and only localized to regions near the corona radiata and thalamic radiation. For MD there were similar trends to the HC comparison: internal capsule, external capsule, cerebral peduncle and superior fronto-occipital fasciculus were significantly higher in LPVD when compared to HC and to IBS. **Conclusions:** The significant group-related microstructural differences in deep gray matter structures included regions that are associated with sensorimotor tasks and cognitive-emotional tasks, suggesting increased processing and modulation of sensory and endogenous pain modulatory systems in LPVD. While IBS and LPVD brains share similar characteristics, the microstructural reorganization in LPVD is more extensive.

Funding: Supported by NIH grants: P30 DK041301, R01 DK048351, R01 HD076756, P50DK64539, Pilot scans were provided by the Ahmanson-Lovelace Brain Mapping Center, UCLA

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