Gail and Gerald Oppenheimer Family Center for Neurobiology of Stress

10th Year Anniversary Symposium of UCLA Specialized Center of Research
Center for Neurovisceral Sciences and Women’s Health

February 9, 2012
Neuroscience Research Building
Contributors

Symposium Chairs

Million Mulugeta, DVM, PhD
Kirsten Tillisch, MD

Sponsors

We gratefully acknowledge the support for this meeting received from:

UCLA Brain Research Institute
UCLA Division of Digestive Diseases
VA Greater Los Angeles Healthcare System

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Gerald Oppenheimer Foundation

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Program

10TH YEAR ANNIVERSARY SYMPOSIUM OF UCLA SCOR
Center for Neurovisceral Sciences and Women’s Health

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

With the generous support from the UCLA Brain Research Institute,
the UCLA Division of Digestive Diseases, the VA Greater Los Angeles
Healthcare System and the Gerald Oppenheimer Foundation

Thursday, February 9, 2012
NEUROSCIENCE RESEARCH BUILDING AUDITORIUM

8:05 am - 8:15 am INTRODUCTION

Symposium Chairs: Kirsten Tillisch, MD (Oppenheimer Center for Neurobiology of Stress; CURE: Digestive Diseases Research Center, UCLA) and Million Mulugeta, DVM, PhD (CURE: Digestive Diseases Research Center; Oppenheimer Center for Neurobiology of Stress, UCLA; VA Greater Los Angeles Healthcare System)

John Mazziotta, MD, PhD
Executive Vice Dean, David Geffen School of Medicine and Associate Vice Chancellor, UCLA

Gary Gitnick, MD
Chief, Division of Digestive Diseases, UCLA

8:15 am - 8:45 am OVERVIEW OF ACCOMPLISHMENTS OF UCLA SCOR IN RESEARCH, CAREER DEVELOPMENT AND OUTREACH

Emeran Mayer, MD
Director, Oppenheimer Center for Neurobiology of Stress; Co-Director, CURE: Digestive Diseases Research Center; Division of Digestive Diseases, UCLA

8:45 am - 8:55 am Frank Hamilton, MD, MPH
Chief, Digestive Diseases Programs Branch, NIDDK, NIH

8:55 am - 9:05 am Janine Clayton, MD
Acting Director, Office of Research on Women’s Health, NIH
9:05 am - 10:05 am  CAREER DEVELOPMENT THROUGH UCLA SCOR (10-minute presentation with 5-minute discussion each)

Chairs: Bruce Naliboff, PhD (Co-Director, Oppenheimer Center for Neurobiology of Stress; CURE: Digestive Diseases Research Center, UCLA; VA Greater Los Angeles Healthcare System) and Margaret Heitkemper, RN, PhD (Department of Biobehavioral Nursing and Health Systems, University of Washington)

Sex Differences in Brain Structure
Eileen Luders, PhD
Laboratory of Neuro Imaging (LONI), Department of Neurology, UCLA

Differential Effects of Sucrose Ingestion on Nucleus Tractus Solitarius Intrinsic Activity in Lean and Obese Women
Lisa Kilpatrick, PhD
Oppenheimer Center for Neurobiology of Stress, Department of Medicine, Division of Digestive Diseases, UCLA

Functional Brain Mapping of Stress-induced Visceral Hypersensitivity in the Rat
Zhuo Wang, PhD
Keck School of Medicine, Department of Psychiatry and Behavioral Sciences, USC

Different Impact of Visceral Stimuli on Attention in IBS Patients and Healthy Controls
Florian Kurth, MD
Oppenheimer Center for Neurobiology of Stress, Department of Medicine, Division of Digestive Diseases, UCLA

10:05 am - 10:30 am  COFFEE BREAK

10:30 am - 12:15 pm  SCOR OUTREACH: SEX DIFFERENCE AND WOMEN’S HEALTH RESEARCH AT UCLA (15-minute presentation with 5-minute discussion each)

Chairs: Yvette Taché (Co-Director, Oppenheimer Center for Neurobiology of Stress; CURE: Digestive Diseases Research Center, UCLA; VA Greater Los Angeles Healthcare System) and Gautam Chaudhuri, MD, PhD (Executive Chair, Department of Obstetrics/Gynecology, UCLA)

Animal Model of Interstitial Cystitis/Painful Bladder Syndrome
Larissa Rodríguez, MD, PhD
Department of Urology, UCLA

Sex Differences in Obesity and Metabolic Disease: That Second X Chromosome Makes a Big Difference
Art Arnold, PhD
Department of Integrative Biology and Physiology, UCLA

Physiology of Membrane-initiated Estradiol Signaling
Paul Micevych, PhD
Department of Neurobiology, UCLA

Imaging Menstrual Cycle Related Mood and Pain Disorders
Andrea Rapkin, MD
Director, UCLA Pelvic Pain Program, Department of Obstetrics/Gynecology, UCLA
UCLA Women’s Health Center of Excellence
Janet Pregler, MD
Director, Iris Cantor UCLA Women’s Health Center

12:15 pm - 1:45 pm  LUNCH AND POSTER SESSION

1:45 pm - 2:45 pm  SCOR OUTREACH: INTER-SCOR COLLABORATION - EARLY LIFE ANTECEDENTS OF CHRONIC DISEASE (20-minute presentation with 10-minute discussion each)

Chairs: Fawzy Fawzy, MD (Louis Jolyon West Distinguished Professor of Psychiatry; Executive Vice Chair, Department of Psychiatry and Biobehavioral Sciences; Executive Associate Director, Semel Institute for Neuroscience and Human Behavior, UCLA) and Michelle Craske, PhD (Director, Anxiety Disorders Research Center; Professor and Vice-Chair, Department of Psychology, UCLA)

Fetal Hormonal Programming of the Brain: Implications for Understanding Sex Differences in Depression
Jill Goldstein, PhD
Director, Harvard SCOR; Professor of Psychiatry and Medicine, Harvard Medical School

Early Life Stress and Glucocorticoid Receptor Gene Methylation in Irritable Bowel Syndrome
Lin Chang, MD
Co-Director, Oppenheimer Center for Neurobiology of Stress; CURE: Digestive Diseases Research Center, Division of Digestive Diseases, UCLA

2:45 pm - 3:00 pm  Joseph Pisegna, MD (Chief, Division of Gastroenterology and Hepatology, VA Greater Los Angeles Healthcare System; Assistant Director, UCLA Affiliated Training Programs in Gastroenterology, UCLA)
Poster Award

Lin Chang, MD
Closing Comments

3:00 pm  END OF SYMPOSIUM

Studies presented at the symposium have been performed at the University of California, Los Angeles; VA Greater Los Angeles Healthcare System; University of Southern California; and Harvard University
Abstracts of Presentations

Symposium Chairs: Kirsten Tillisch, MD (Financial Disclosure: None)
Million Mulugeta, DVM, PhD (Financial Disclosure: None)

OVERVIEW OF ACCOMPLISHMENTS OF UCLA SCOR IN RESEARCH, CAREER DEVELOPMENT AND OUTREACH

Emeran Mayer, MD
Director, Oppenheimer Center for Neurobiology of Stress; Co-Director, CURE: Digestive Diseases Research Center; Division of Digestive Diseases, UCLA
* Financial Disclosure: None

CAREER DEVELOPMENT THROUGH UCLA SCOR

Chairs: Bruce Naliboff, PhD (Financial Disclosure: None)
Margaret Heitkemper, RN, PhD (Financial Disclosure: None)

Sex Differences in Brain Structure

Eileen Luders, PhD
Laboratory of Neuro Imaging (LONI), Department of Neurology, UCLA

Differences in cortical gray matter, cortical thickness and cortical complexity between men and women will be discussed.
* Financial Disclosure: None

Differential Effects of Sucrose Ingestion on Nucleus Tractus Solitarius Intrinsic Activity in Lean and Obese Women

Lisa Kilpatrick, PhD
Oppenheimer Center for Neurobiology of Stress, David Geffen School of Medicine, Department of Medicine, Division of Digestive Diseases, UCLA

Background: The study of intrinsic fluctuations in the blood oxygen-level dependent (BOLD) signal of functional magnetic resonance imaging (fMRI) can provide insight into the effect of physiological states on brain processes. Using this tool, the present study aimed to determine if ingestion of a sweetened beverage results in differential engagement of central vagal pathways in lean and obese women.

Methods: In a two-day double-blind crossover design, 11 lean and 11 obese healthy women underwent fMRI scanning following ingestion of two beverages of different sucrose content, but identical sweetness. During scans, subjects rested with eyes closed.

Results: BOLD fluctuations demonstrated a shift towards higher frequencies following sucrose ingestion (compared to the artificially sweetened drink) in nucleus tractus solitarius (NTS) for both groups. For lean but not obese women, greater NTS high frequency power was associated with decreased hunger ratings. For obese but not lean women, greater NTS high frequency power was associated with greater functional connectivity of NTS with regions of a central reward network.

Conclusions: These findings demonstrate sucrose related changes in oscillatory dynamics of BOLD fluctuations in a key brain region involved in food homeostasis (NTS). The increase in NTS high frequency power following ingestion of sucrose may reflect an increase in intrinsic neural activity due to vagal stimulation associated with the presence of nutrients in the intestinal lumen. In addition, the results revealed differences...
between lean and obese subjects providing additional support for altered interaction between homeostatic and reward networks in obese individuals.

* Financial Disclosure: None

Functional Brain Mapping of Stress-induced Visceral Hypersensitivity in the Rat

Zhuo Wang, PhD
Keck School of Medicine, Department Psychiatry and Behavioral Sciences, USC

**Background:** Chronic water avoidance stress (WAS) induces sustained visceral hyperalgesia in rats measured as enhanced visceromotor response (VMR) to colorectal distension (CRD). This model incorporates two characteristic features of human irritable bowel syndrome (IBS), visceral hyperalgesia and a prominent role of stress in the onset and exacerbation of IBS symptoms.

**Aim:** To further validate WAS as a model for IBS, we used an autoradiographic perfusion method to map the brain correlates of WAS-induced visceral hyperalgesia and compared the results with human brain imaging findings that characterize differences in functional brain responses to CRD between IBS patients and healthy controls.

**Methods:** Male rats were implanted with telemetry transmitters to measure CRD-induced abdominal electromyography and cannulated for IV injection. Rats were exposed to 1-hr WAS or sham treatment for 10 days. VMR to CRD was measured before and after the treatment. Cerebral blood flow (CBF) mapping was performed by IV injection of radiotracer ([14C]-iodoantipyrine) while the rat was receiving either 60- or 0-mmHg CRD, followed by rapid euthanasia. Regional CBF-related tissue radioactivity was quantified in autoradiographic images of brain slices and analyzed in 3-dimensionally reconstructed brains by statistical parametric mapping.

**Results:** Rats exposed to WAS showed significant increase in VMR to CRD compared to baseline (P<0.001). Compared to sham controls, stressed rats showed greater CRD-induced activation in the insular cortex and amygdala, but reduced activation in the prelimbic area of medial prefrontal cortex (mPFC). Functional connectivity analysis revealed anticorrelation between the mPFC and amygdala in controls receiving 60-mmHg CRD, but not in stressed rats.

**Conclusions:** Stress-induced visceral hyperalgesia was associated with altered activation patterns in brain areas concerned with interoceptive processing and emotional arousal. As similar differences in brain correlates of visceral pain have been reported between IBS patients and healthy controls, these findings provide support for the face and construct validity of the chronic WAS model for IBS. The absence of mPFC-amygdala anticorrelation in stressed rats suggests impaired cortical modulation of the emotional arousal network as a central mechanism underlying stress-induced visceral hyperalgesia.

* Financial Disclosure: None

Different Impact of Visceral Stimuli on Attention in IBS Patients and Healthy Controls

Florian Kurth, MD
Oppenheimer Center for Neurobiology of Stress, David Geffen School of Medicine, Department of Medicine, Division of Digestive Diseases, UCLA

Chronic abdominal pain is the hallmark of patients with Irritable Bowel Syndrome (IBS). These patients are known to show heightened attention to interoceptive signals from the gut. One would therefore expect IBS patients to be more easily distracted by interoceptive perceptions. It remains largely unknown, however, if this is the case. It is furthermore unclear, if processing of an emotionally neutral attention task is the same in IBS patients and healthy controls. To address these questions, 20 patients with IBS and 20 healthy controls matched for sex and age underwent two fMRI experiments. Both were centered on a letter search task with two levels of difficulty (high and low load). The first experiment added subliminal (i.e. below perception threshold) rectal distensions to this attention task, while the second one added supraliminal (i.e. mild) rectal distensions as potentially distracting interoceptive stimuli. In the first experiment, patients with IBS showed activation of more widespread brain regions during the attention task compared to controls, particularly for high load tasks. The subliminal rectal distensions did not interfere with the task. In the second experiment, patients with IBS exhibited less activation in widespread brain regions compared to controls – more so during
the high load task. During low load tasks, mild rectal distensions caused significantly less activation in both patients and controls, with no group difference. During high load tasks, however, significantly less activation with mild rectal distension was observed in patients only. We interpret the more widespread activations during subliminal distraction as generally increased attention in absence of interoceptive perception. The strikingly lower activation in IBS during mild rectal distensions may be interpreted as a diminished focus on the task in the presence of interoceptive perception. Particularly the different impact of mild rectal distensions during the high load task may reflect a deficit to suppress distracting information from the viscera in IBS patients.

* Financial Disclosure: None

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**SCOR OUTREACH: SEX DIFFERENCES AND WOMEN'S HEALTH RESEARCH AT UCLA**

**Chairs:** Yvette Taché, PhD (Financial Disclosure: Research grant from Kirin Holdings Co. Ltd)
Gautam Chaudhuri, MD, PhD (Financial Disclosure: None)

**Animal Model of Interstitial Cystitis/Painful Bladder Syndrome**

**Larissa Rodriguez, MD**
Department of Urology, UCLA

Psychological stress can exacerbate functional pain disorders, such as interstitial cystitis/painful bladder syndrome (IC/PBS), a visceral pain disorder of the lower urinary tract thought to result from neuronal dysregulation. Current existing animal models of IC/PBS involve intravesical use of inflammatory agents with limited translational relevance for the human condition. We sought to mimic some of the epidemiological aspects of human IC/PBS including vesical hyperalgesia and urinary frequency associated with stress exacerbations and other co-morbid conditions such as IBS. We have evaluated the role of glutamate (Glu) as the primary excitatory neurotransmitter in the central nervous system (CNS) in spinal astroglia in a model of chronic psychological stress–induced visceral hyperalgesia in female Wistar-Kyoto (WKY) rats. This rodent WAS model represents a novel tool for studying syndromes of lower urinary tract dysfunction and pain. Repeated psychological stress results in changes in voiding behaviors and increased global pain in response to physiologic stimuli, associated with alterations in glutamate processing in the CNS. Early results suggest that manipulation of Glu handling may be able to inhibit or reverse the allodynia and visceral hyperalgesia developing as a consequence of psychological stress.

* Financial Disclosure: None

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**Sex Differences in Obesity and Metabolic Disease: That Second X Chromosome Makes a Big Difference**

**Arthur Arnold, PhD**
Department of Integrative Biology and Physiology, UCLA

Obesity represents a risk factor for many types of metabolic disease, including diabetes, coronary heart disease, osteoarthritis, and even cancer. Men and women show significant sex differences in adiposity and co-morbid diseases. The Reue and Arnold labs at UCLA have studied body weight, adiposity, and metabolic parameters in Four Core Genotypes (FGC) mice, in which sex chromosome complement (XX vs. XY) is independent of gonadal sex. The model produces XX and XY gonadal males (XXM, XYM), and XX and XY gonadal females (XXF, XYF), and is useful for discriminating the roles of gonadal hormones and sex chromosomes which cause sex differences in traits. Adult male C57BL/6 mice weigh about 25% more than adult females, and the difference is abolished after gonadectomy of adults, indicating that the sex difference is caused by gonadal secretions. In gonadally intact FCG mice, however, XX mice of either gonadal sex weigh 6-9% more than XY mice. After gonadectomy of adults, XX mice eating low fat chow slowly gain more weight and fat, and by 10 months after gonadectomy XX mice have nearly twice the body fat of XY mice. Our extensive metabolic studies of these mice suggest that the greater adiposity of XX mice is because they eat more than XY mice after gonadectomy. Study of progeny of C57BL/6 XY* mice, in which the number of X and Y chromosomes is varied independently, indicates that it is the number of X chromosomes that causes the
sex chromosome effect, and the Y chromosome appears to have little effect. The X chromosome effect is exacerbated in mice eating a high fat diet. Several candidate X genes thought to escape X-inactivation are expressed at a higher level in metabolic tissues (fat, liver) in gonadectomized XX mice relative to XY mice. These results indicate that the dose or imprint on X genes dramatically affects adiposity, and the X gene effects interact with gonadal hormone effects to cause sex differences in adiposity.

Supported by NIDDK DK083561 grant to AP Arnold, Xuqi Chen, and Karen Reue.

* Financial Disclosure: None

**Physiology of Membrane-initiated Estradiol Signaling**

**Paul Micevych, PhD**  
Department of Neurobiology, UCLA

Over the decades, our understanding of estrogen receptor (ER) function has evolved. Although there are a number of candidates that mediate estradiol membrane signaling (EMS), our results point to the nuclear ERs, ERα and ERβ, as the most promising. These classical intracellular receptor proteins are trafficked to the membrane and interact with metabotropic glutamate receptor (mGluR) to activate phospholipase C dependent signaling pathways including release of intracellular calcium stores and stimulation of the MAPK pathway. An important site of this activation is the arcuate nucleus of the hypothalamus (ARH) where estradiol regulates reproduction and energy balance. In the ARH, ERα transactivation of mGluR1a mediates dendritic morphology inducing sexual receptivity. In more rostral hypothalamic areas, activation of the ERα-mGluR1a complex in astrocytes stimulates neuroprogesterone synthesis, a critical step in estrogen positive feedback of the LH surge. These results expand our understanding of estradiol action in the brain and reveal an integration of hormonal and neural signaling that regulating physiology and behavior.

* Financial Disclosure: None

**Imaging Menstrual Cycle-related Mood and Pain Disorders**

**Andrea Rapkin, MD**  
Director, UCLA Pelvic Pain Program, Department of Obstetrics and Gynecology

Premenstrual dysphoric disorder and dysmenorrhea are two well defined menstrual cycle related mood and pain disorders. This discussion will illustrate how brain imaging can expand our understanding of these syndromes. Both disorders are at the far end of a continuum that has been considered normal menstrual cycle related symptoms. Both are triggered by the rise and fall of sex steroids with ovulation. In the case of severe premenstrual syndrome, termed premenstrual dysphoric disorder, approximately 5 to 8% of reproductive age women are affected, with physical symptoms such as abdominal pain, bloating, and breast tenderness and psychological symptoms, including irritability, depression, anxiety and mood swings that cause significant impairment, in up to 8% of reproductive age women. Symptoms are absent in the prepubertal pregnancy and menopausal years, but persist throughout the reproductive years, for up to to 14 days per month. Significant dysmenorrhea recurs on a monthly basis and affects 25% of women and 90% of teens, with severe distressing symptoms and up to 30% of individuals. Thus these syndromes are important by virtue of their repetitive nature over a prolonged period of time in young women.

The effect on brain functioning has only recently become an important topic of investigation. We studied regional cerebral metabolism across the menstrual cycle and explored the relationship of symptom change to local metabolic change. Although there were no group differences in hormone levels in either the follicular or late luteal phases, women with PMDD, but not comparison subjects showed an increase in cerebellar activity, particularly in the right cerebellum vermis from the follicular phase to the luteal phase. In the PMDD group, this increase cerebella activity was correlated with worsening of mood. Cerebellum has been recently been shown to have a role in behaviors involving emotion, pain and executive functioning. In a second study, we investigated cerebral gray matter volume comparing women with PMDD to asymptomatic women. Women with PMDD had greater gray matter volume than control women in the cerebellum (p = 0.005) but in no other brain structure. The gray matter effects were most extensive in the cerebellar vermis and cerebellar regions previously associated with emotional processing. If time allows, fmr study of PMDD will be discussed.
Dysmenorrheic women have decreased sensory thresholds both within and outside areas of referred menstrual pain. Recent brain imaging studies of women with dysmenorrhea will be discussed. During a painful thermal stimulus, there were no differences in brain activation, however, dysmenorrheic women did not show the widespread deactivation seen in controls, suggesting alterations in resting state networks in dysmenorrheic women (Vincent K 2011). Dysmenorrhea was associated with abnormal metabolic changes in brain regions involved in pain processing; specifically, disinhibition of the orbital frontal network. Brain morphological changes were also associated with cyclical menstrual pain (C. Tu 2009) Menstrual pain severity was correlated with decreased gray matter volume in regions associated with top down modulation of pain and generation of negative effect. Implications of these findings will be discussed.

* Financial Disclosure: None

**UCLA Women’s Health Center of Excellence**

**Janet Pregler, MD**  
Director, Iris Cantor-UCLA Women’s Health Center

The UCLA National Center of Excellence in Women’s Health was founded in 1997 with an award from the Department of Health and Human Services, Office on Women’s Health. Activities of the Center include provision of comprehensive primary and specialty care, research, including maintenance of a clinical research center and a multidisciplinary pilot project funding program, professional education, and community outreach. The Center’s research programs have been successful in promoting women’s health research, multidisciplinary and translational research, and obtaining funding for basic and clinical research in women’s health.

* Financial Disclosure: None

**SCOR OUTREACH: INTER-SCOR COLLABORATION - EARLY LIFE ANTECEDENTS OF CHRONIC DISEASE**

**Chairs:**  
Fawzy Fawzy, MD (Financial Disclosure: None)  
Michelle Craske, PhD (Financial Disclosure: None)

**Fetal Hormonal Programming of the Brain: Implications for Understanding Sex Differences in Depression**

**Jill Goldstein, PhD**  
Director of Research, Connors Center for Women’s Health and Gender Biology; Departments of Psychiatry and Medicine, Harvard Medical School

Women are at twice the risk of major depressive disorder (MDD) than men, a devastating chronic illness with substantial functional disability. We are investigating the hypothesis that the vulnerability for sex differences in MDD begins with the fetal disruption of hypothalamic pituitary adrenal (HPA) axis development (i.e., prenatal stress model), that involves brain regions that are highly sexually dimorphic. We are testing the hypothesis that fetal HPA disruption will be significantly related to sex differences in stress response circuitry deficits, endocrine abnormalities and mood dysregulation in adulthood. In a 50-year cohort study of offspring of mothers followed through pregnancy and sera stored at NIH, using functional brain imaging, we demonstrated sex-specific stress response circuitry brain activity deficits associated with endocrine disruptions in adult offspring with MDD compared with healthy adult offspring. Sex-specific brain activity deficits in MDD in adulthood were significantly associated with maternal prenatal immune activation abnormalities, assessed in prenatal sera. Findings suggest fetal hormonal programming of sex differences in MDD may, in part, be associated with disruption of the maternal immune response resulting in sex-specific effects on offspring’s HPA circuitry, expressed in adulthood as brain activity and endocrine deficits. Thus, the fetal hormonal programming of the stress response circuitry may be important for understanding vulnerability to MDD, particularly in women.

* Financial Disclosure: None
Early Life Stress and Glucocorticoid Receptor Gene Methylation in Irritable Bowel Syndrome

Lin Chang, MD
Co-Director, Oppenheimer Center for Neurobiology of Stress; CURE: Digestive Diseases Research Center, Division of Digestive Diseases, UCLA

Early adverse life events (EALs), such as abuse and other traumatic events, results in alterations in stress-responsive neurobiological systems, which in turn, increase the vulnerability of developing long-term health, behavioral and social problems. Childhood and adult abuse have been shown to be more prevalent among patients with irritable bowel syndrome (IBS) than healthy individuals. We recently demonstrated that different types of EALs before an age of 18 years were more prevalent among IBS patients. Compared to healthy controls (n=435, 77% women), IBS patients (n=294, 79% women) had a higher prevalence of general trauma (78.5% vs 62.3%), physical punishment (60.6% vs 49.2%), emotional abuse (54.9% vs 27.0%), and sexual events (31.2% vs 17.9%) (all $p$'s <.001). These significant differences were mainly observed in women. Of the EAL domains, emotional abuse was the strongest predictor of IBS ($P$<0.001). Although EALs and psychological variables were related, EALs had an independent association with IBS ($P$=0.04).

In rodent studies, early life stress has been linked to increased methylation of the glucocorticoid receptor (GR) promoter in the hippocampus, resulting in decreased GR expression and feedback inhibition of the hypothalamic-pituitary-adrenal (HPA) axis by glucocorticoids and enhanced HPA axis response to stress. We found that in both IBS and control groups, a history of EALs was associated with a greater cortisol response to a visceral stressor than individuals without EALs. These findings support a role for EALs in the development and life-long functioning of the HPA axis. We recently compared the methylation status of CpG sites in the promoter region of NR3C1 (GR) gene isolated from peripheral blood T lymphocytes in IBS patients and healthy controls. NR3C1 methylation was measured in 23 IBS patients (n=23, 9M,14F; 6 IBS-C, 9 IBS-D, and 8 IBS-M) and 23 controls (n=23, 9M,14F). NR3C1 methylation was not present at any of the CpG sites in any of the controls. In contrast, all but 1 IBS patient had methylation of the NR3C1. A history of EALs was associated with increased % NR3C1 methylation. Methylation pattern was also bowel habit specific. IBS-D patients showed a higher % methylation at CpG sites 1-3 (p=0.003), while IBS-C patients had greater methylation at CpG sites 7-8 (p=0.003). mRNA expression of the active isoform of NR3C1 (GRα) was significantly lower in IBS vs. controls (p=0.002) and negatively correlated with % NR3C1 methylation (-0.50, r=0.001). While lower mRNA levels of GRα were associated with a greater cortisol response in IBS, they were associated with a lower cortisol response in controls. These findings demonstrate for the first time that epigenetic changes involving the GR promoter (NR3C1) are associated with the symptom complex of IBS. NR3C1 methylation pattern is associated with bowel habit predominance and a history of EALs, which supports a link between early life psychosocial exposure and long-lasting programming of gene expression. NR3C1 methylation is associated with decreased GRα mRNA expression, which may contribute to the increased stress responsiveness in IBS. NR3C1 methylation is a potential biomarker for IBS.


* Financial Disclosure: None
Biosketches of Speakers

Arthur Arnold, PhD
Department of Integrative Biology and Physiology, UCLA

Arthur P. Arnold, PhD, studies mechanisms causing sexual differences in physiology and disease. His research has included the discovery of large sexual dimorphisms in the brain, development of several animal models for studying sex differences, and studies of mechanisms by which sex-biasing factors operate. He received his PhD in neurobiology and behavior from the Rockefeller University.

Dr. Arnold is currently Distinguished Professor in the Department of Integrative Biology & Physiology at UCLA. He has held positions as departmental Chair, Associate Director of the UCLA Brain Research Institute, and Chair of the UCLA interdepartmental PhD and undergraduate programs for Neuroscience. He is Director of the UCLA Laboratory of Neuroendocrinology.

Dr. Arnold was Inaugural President of the Society of Behavioral Neuroendocrinology, and is a fellow of the AAAS and the John Simon Guggenheim Memorial Foundation. He has served on editorial boards several journals including *Hormones and Behavior*, *Journal of Neurobiology*, and *Developmental Neuroscience*. He is the Editor-in-Chief of *Biology of Sex Differences*, the official journal of the Organization for the Study of Sex Differences.

Lin Chang, MD
Co-Director, Oppenheimer Center for Neurobiology of Stress, CURE: Digestive Diseases Research Center, Department of Medicine, Division of Digestive Diseases, 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 Center for Neurobiology of Stress at the David Geffen School of Medicine at UCLA. Dr. Chang earned her medical degree from the UCLA School of Medicine and completed her internship and residency in internal medicine at Harbor-UCLA Medical Center. She completed her gastroenterology fellowship training at the UCLA Affiliated Training Program in Gastroenterology. Dr. Chang’s clinical expertise is in functional gastrointestinal disorders which include irritable bowel syndrome (IBS), chronic constipation, and functional dyspepsia. Dr. Chang’s research is focused on the pathophysiology of IBS related to stress, sex differences, and neuroendocrine alterations and the treatment of IBS. She is a funded NIH-investigator studying the central and peripheral mechanisms underlying IBS. She is the recipient of the Janssen Award in Gastroenterology for Basic or Clinical Research and the AGA Distinguished Clinician Award. Dr. Chang has authored more than 70 original research articles, 48 review articles, and 19 book chapters on her specialty interests and is a frequent speaker at national and international meetings. She is a fellow of the American Gastroenterological Association and an active member of several professional societies, including the American College of Gastroenterology and the Society for Neuroscience. She is also a member of the Rome Foundation Board of Directors. She is the President Elect in the newly merged American Neurogastroenterology and Motility Society-Functional Brain Gut Research Group (ANMS-FBG).

Jill M. Goldstein, PhD
Director of Research, Connors Center for Women’s Health and Gender Biology; Departments of Psychiatry and Medicine, Harvard Medical School

Dr. Goldstein is a Professor of Psychiatry and Medicine, Departments of Psychiatry and Medicine at Harvard Medical School and Director of Research, Connors Center for Women’s Health & Gender Biology at Brigham and Women’s Hospital and Director of Research on Gender Neurobiology and Women’s Mental Health at BWH. She is also a Senior Scientist at Massachusetts General Hospital. Over the last 25 years, she has become an internationally-recognized expert on sex differences in the human brain and how disruption of sexually dimorphic developmental processes can help us understand sex differences in adult psychiatric disorders, such as schizophrenia, affective psychoses, and depression, and the comorbidity of psychiatric
disorders with general medicine, such as cardiovascular disease. She has published numerous articles in these areas and has received numerous NIH awards to pursue this work. Her program of research, called the Clinical Neuroscience Laboratory of Sex Differences in the Brain (http://cnl-sd.bwh.harvard.edu) consists of an interdisciplinary team of investigators, integrating structural and functional brain imaging studies, psychophysics, neuroendocrine studies of hormones and brain function, genetics, inflammatory factors, and collaborative efforts with animal investigators studying genes, hormones, inflammation and the brain [e.g. http://mddsccor.bwh.harvard.edu ]. Brain circuitries under current investigation include the stress response circuitry, memory and working memory (and brain aging of these circuitries), and reward circuitry implicated in the neural circuitry of obesity. She was named the 2007 Spinoza Professor by the Academic Medical Center, University of Amsterdam for her work on the role of hormones, sex differences and the brain for understanding clinical disorders in medicine. Dr. Goldstein is also building a unique research infrastructure for the Connors Center and Department of Psychiatry at Brigham & Women’s Hospital to foster collaborative efforts to understand mechanisms that explain sex differences in health and disease across disciplines and methods of study and to provide a source of knowledge and training for future young scientists and clinicians in women’s health and gender biology.

Lisa Kilpatrick, PhD
Oppenheimer Center for Neurobiology of Stress, David Geffen School of Medicine, Department of Medicine, Division of Digestive Diseases, UCLA

Lisa Kilpatrick completed a PhD degree in Biological Sciences from the University of CA, Irvine. Dr. Kilpatrick is part of the neuroimaging and psychophysiology cores at the Center for Neurobiology of Stress. Her research focuses on the altered central nervous system processes in functional pain disorders such as Irritable Bowel Syndrome (IBS) and in other patient populations thought to have altered interoceptive processing such as obesity. She is dedicated to exploring sex differences in nervous system processes as an important step towards tailoring therapies to individual neurobiologies. Current projects include sex differences in genetic and neural pathways influencing IBS pathophysiology.

Florian Kurth, MD
Oppenheimer Center for Neurobiology of Stress, David Geffen School of Medicine, Department of Medicine, Division of Digestive Diseases, UCLA

Florian Kurth is a postdoctoral scholar at the Center for Neurobiology of Stress at UCLA. He graduated from Medical School in Duesseldorf, Germany in 2008 and received his doctoral degree on “Structure-Function Relationships in the Human Insular Cortex” in 2010. After coming to UCLA in 2010, he joined the Center for Neurobiology of Stress in June 2011. His research focuses on mind-body interactions using structural and functional neuroimaging.

Eileen Luders, PhD
Laboratory of Neuro Imaging (LONI), Department of Neurology, UCLA

Dr. Eileen Luders received her Bachelors and Masters degrees in Psychology from the University of Magdeburg, Germany followed by a PhD, summa cum laude, in Neuropsychology from the University of Zurich, Switzerland. She is now an Assistant Professor at the UCLA Laboratory of Neuro Imaging (LONI). Her research interests are in sexual dimorphism of the human brain, brain morphology in meditation practitioners, and neuroanatomical correlates of intelligence.

Paul Micevych, PhD
Department of Neurobiology, UCLA

Dr. Micevych is a neuroendocrinologist. He received his doctorate from the University of Minnesota and did his postdoctoral training in the Department of Neurologic Surgery at the Mayo Clinic. Dr. Micevych is a Professor in the Departments of Neurobiology and Head & Neck Surgery. He is a member of the UCLA
January Pregler, MD
Director, Iris Cantor-UCLA Women's Health Center

Janet P. Pregler, M.D. is a nationally recognized educator and advocate in women's health. A faculty member at the David Geffen School of Medicine since 1992, she was named director of the Iris Cantor-UCLA Women's Health Center in 1997. She is also director of the UCLA National Center of Excellence in Women's Health, established by a contract awarded by the Department of Health and Human Services, Office on Women's Health, also in 1997. The Iris Cantor-UCLA Women's Health Center is one of only nine academic women's health programs in the country to complete this program and be designated an "Ambassador for Change", charged with disseminating models to address inequities in the care of women nationally.

Dr. Pregler is co-editor of the textbook "Women's Health: Principles and Clinical Practice". She has developed educational programs on women's health for the American College of Physicians, Centers for Disease Control and Prevention, National Heart, Lung, and Blood Institute, and the Department of Health and Human Services, Office on Women's Health. Under her leadership, the Iris Cantor-UCLA Women's Health Center has developed nationally recognized educational programs for physicians, nurses, and community members on the prevention of coronary artery disease. Dr. Pregler worked with a group of women philanthropists to establish the Executive Advisory Board Iris Cantor-UCLA Women's Health Center pilot project fund, which gives yearly grants to UCLA researchers to study women's health topics, including heart disease, stroke, cancer, the effect of stress on the immune system, interventions such as plant-derived substances and Yoga to improve women's health, and social issues that affect health, such as human trafficking.

Dr. Pregler has received numerous honors and awards for her work, including the Award for Excellence in Medical Education of the David Geffen School of Medicine at UCLA, a "Commendation" by the County of Los Angeles for work on the Women's Health Policy Council, County of Los Angeles Department of Health Services, a "Certificate of Recognition" for contributions to the Los Angeles County-Office of Women's Health Cervical Cancer Prevention and Education Initiative by the California Legislature Assembly, a "Certificate of Special Congressional Recognition" for work as a member of the Women's Health Policy Council, Los Angeles County Office of Women's Health, by Hilda L. Solis, Member of Congress, a "Certificate of Appreciation", for leadership in the National Centers of Excellence in Women's Health program, Office on Women's Health, Department of Health and Human Services, and a "Certificate of Recognition", for work in the area of women's health, from the State of California Senate.

Dr. Pregler completed medical school at Northwestern University, and a residency in primary care internal medicine at George Washington University. She is board certified in internal medicine.

Andrea Rapkin, MD
Director, UCLA Pelvic Pain Program, Department of Obstetrics and Gynecology

Andrea J. Rapkin, M.D. is a Professor of Obstetrics and Gynecology at the UCLA David Geffen School of Medicine. Dr. Rapkin received her undergraduate degree from Cornell University and medical degree from State University of New York at Buffalo, and completed residency in Obstetrics and Gynecology at UCLA. Dr. Rapkin is Director of the UCLA Pelvic Pain Clinic and of the UCLA Premenstrual Disorders Program. Her research has focused on understanding the etiology and management of PMS/PMDD and of chronic pelvic pain disorders including endometriosis and vulvodynia.

Dr. Rapkin is a member of the Society for Gynecologic Investigation, the American Pain Society, the International Association for the Study of Pain, the International Society of Psycho-Endocrinology and is on
Dr. Larissa V. Rodríguez is an Associate Professor and Co-Director of the Division of Female Urology, Reconstructive Surgery, and Urodynamics and Director of Female Urology Research at the University of California Los Angeles (UCLA) School of Medicine. She obtained a Bachelors of Science degree in Mathematics from the Massachusetts Institute of Technology and completed her medical training and Urology residency at the Stanford University School of Medicine. She then served as a Fellow in Female Urology, Voiding Dysfunction, and Reconstructive Surgery at the David Geffen School of Medicine at UCLA prior to joining the faculty in 2001.

Dr. Rodríguez’ clinical research focuses on outcomes of vaginal and robotic surgery, quality of life as it relates to pelvic floor disorders, and the pathophysiology and treatment of interstitial cystitis. In the laboratory she is pursuing investigations in the role of stress on urinary symptoms. She has been a recipient of numerous research grants and has served as reviewer of multiple journals and as ad hoc reviewer for the NIH and other research foundations. She has published numerous research articles and book chapters and is co editor of the 3rd Edition of Female Urology, Raz S. and Rodríguez L.V. (editors), from W. B. Sounders Publishing Company. As a clinician she has been involved in the education and mentorship of Female Urology fellows since 2001. As a researcher she has mentored numerous residents, fellows and junior faculty as has been the recipient of multiple research awards from the American Urological Association (AUA), the Western Section of the AUA, and the Society of Urodynamics and Female Urology (SUFU). In 2008 she was the recipient of the Zimskind Award, an award given by SUFU to an individual with significant contribution to the field of Pelvic Medicine and Voiding Dysfunction within the first 10 years of their career.

Zhuo Wang, PhD
Keck School of Medicine, Department of Psychiatry and the Behavioral Sciences, USC

Zhuo Wang is a Research Assistant Professor in the Department of Psychiatry and the Behavioral Sciences at USC. He earned his BS degree in Biology at the University of Science and Technology of China, and PhD in Neurobiology at the University of Southern California. 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 anxiety and functional pain disorders, with an emphasis on understanding the brain mechanisms underlying sex-related differences, modulation by stress, and pharmacological intervention. His main approach is autoradiographic blood flow mapping in unrestrained rodents, performed in parallel with other physiological and behavioral measurements.
1. Pro-inflammatory Cytokines and Spinal Glia Markers in the Model of Chronic Water Avoidance Stress: A Time Course Study

S Bradesi, V Golovatscka, H Ennes, EA Mayer

UCLA Oppenheimer Center for Neurobiology of Stress

Background: Chronic water avoidance stress (WAS) has been established as a model of persistent stress-induced visceral hyperalgesia in the rat and recent evidence suggests an important role of spinal glia activation in this sensitization (Am J Physiol 301, 2011). However, the mechanism(s) underlying the development of this spinal neuroimmune activation remain to be determined.

Aims: To assess the time course of WAS-induced changes in peripheral and spinal pro-inflammatory cytokines, as well as glia markers in the spinal cord and dorsal root ganglia (DRGs), and to identify possible relationship of peripheral and central immune activation.

Method: Blood, lumbar spinal cord and DRG samples were collected from the following groups of male adult Wistar rats: i) controls, ii) single exposure to 1hr WAS (collection 1hr and 24hrs post WA), iii) exposure to 5 WA sessions (collection 24hrs after the last WA), and iiii) exposure to daily 1hr WA session for 10 consecutive days, collection 24 hrs post WA. The following markers were assessed: the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α (ELISA), the astrocytic marker GFAP (glial fibrillary acidic protein), connexin 43 (a tight junction protein involved in the network of astrocytes) and the microglia marker Iba1 (Western blotting). Blood spinal cord barrier (BSCB) permeability in all groups was assessed by Evans Blue extravasation into the spinal cord after intravenous injection.

Results: Circulating levels of IL-1β were elevated 1 and 24 hrs after 1 WA stress session, and 24hrs after 5 WA, compared with controls (P<0.05). Neither circulating IL-6 nor TNF-α showed significant changes. Spinal IL-1β and IL-6 were increased 1hr after 1 WA while TNF-α was increased 24hrs post 1 WA, compared with controls (P<0.05). Both spinal GFAP and connexin 43 were significantly increased 24hrs after 5 WA and decreased after 10 WA (P<0.05) while Iba1 showed no significant change across the different time points. In DRGs, GFAP was increased 24hrs after 10 WA while connexin 43 was increased 24hrs after 5 WA. Evans blue was increased 24hrs after 5 WA sessions only, suggesting repeated stress-induced increased BSCB permeability.

Conclusions: Our results suggest neuroimmune activation in DRG and spinal cord during WAS. The temporal association of a single stress-induced IL-1β increase in plasma and spinal cord suggest a possible role of early cytokine mediated signaling from the periphery to the CNS. Activation of spinal astrocytes and DRG satellite glia activation was only observed after repeated stress exposure, which may be in part be related to changes in BSCB permeability. Further studies are required to assess the role of circulating and peripheral pro-inflammatory mediators in stress-induced visceral hyperalgesia.

2. Acute Psychological Stress Induces an Immediate Naloxone-independent Visceral Analgesia to Colorectal Distension in Mice

M Larauche, H Duboc, A Mulak, Y Taché

UCLA Oppenheimer Center for Neurobiology of Stress and CURE VA GLA HS, Los Angeles CA, USA

Background: Stress can elicit somatic analgesia also known as stress-induced somatic analgesia (SISA). Both opioid and non-opioid forms of SISA have been identified. We recently reported in mice that repeated exposure to water avoidance stress (WAS) affects the visceromotor response (VMR) to colorectal distension (CRD) differentially depending on the method used to monitor visceral sensitivity: WAS induces stress-
induced visceral analgesia (SIVA) 24h post stress in animals tested for visceral pain non invasively while it leads to visceral hyperalgesia in animals equipped with the traditional method of EMG.

Aims: To determine whether WAS in its acute form induces SIVA in male mice and to assess whether the analgesic response is opiate dependent.

Methods: Male C57Bl/6 mice (23-33 g, 1-4/cage) were monitored for VMR to CRD (15, 30, 45 and 60 mmHg, 3 times each, 10 sec, 4 min interval) using a novel non-invasive technique. The 1st CRD (day 0) served as baseline VMR, then mice were exposed to 1h WAS on day 1. Two groups were injected subcutaneously (sc) with naloxone (1 mg/kg) (n=6) or saline (0.1 ml) (n=8) 10 min before WAS, another control group (n=12) received no injection. The 2nd CRD was monitored 45-50 min after WAS. The VMR to CRD after WAS was expressed as percentage of the respective baseline values at different pressures of CRD. Defecation was monitored after each WAS session. VMR data were analyzed using 2-way ANOVA and Bonferroni post-hoc test, defecation data by unpaired t test.

Results: Immediately after the 1h-WAS, non-injected mice exhibited a visceral analgesia at 60 mmHg (VMR: 78.9 ± 7.2 vs 100.0 ± 0.0%, p<0.05). Compared to non injected mice, saline or naloxone sc injections before WAS enhanced the visceral analgesia inducing an analgesic response at 30, 45 and 60 mmHg for saline and at 45 and 60 mmHg for naloxone (Table). Compared to non injected mice, WAS-induced defecation was reduced by sc injection of saline (4.2 ± 1.3 vs 6.8 ± 0.5 pellets/h/20g body weight, p<0.05) but not naloxone (8.0 ± 2.7 vs 6.8 ± 0.5 pellets/h/20g body weight p>0.05).

Conclusions: Exposure to an acute psychological stress induces an immediate opiate-independent visceral analgesia in male mice. Subcutaneous injection potentiates the immediate analgesic response suggesting that the concomitant acute somatic noxious stimulus may exert heterotopic analgesic effects.

Supported by NIH P50 DK-64539 (YT), 1K01DK088937 (ML), SNFGE (HD) and APHP (HD).

<table>
<thead>
<tr>
<th>CRD (mmHg)</th>
<th>Non injected</th>
<th>Post WAS</th>
<th>Saline SC</th>
<th>Post WAS</th>
<th>Naloxone SC</th>
<th>Post WAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>Post WAS</td>
<td>baseline</td>
<td>Post WAS</td>
<td>baseline</td>
<td>Post WAS</td>
</tr>
<tr>
<td>30</td>
<td>48.2 ± 7.7</td>
<td>37.5 ± 5.1</td>
<td>52.6 ± 11.1</td>
<td>18.9 ± 6.7**</td>
<td>41.4 ± 9.2</td>
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<tr>
<td>45</td>
<td>80.0 ± 7.1</td>
<td>66.9 ± 6.8</td>
<td>81.5 ± 8.8</td>
<td>55.9 ± 7.9*</td>
<td>89.7 ± 9.0</td>
<td>43.7 ± 8.3***</td>
</tr>
<tr>
<td>60</td>
<td>100.0 ± 0.0</td>
<td>78.9 ± 7.2*</td>
<td>100.0 ± 0.0</td>
<td>69.8 ± 7.8*</td>
<td>100.0 ± 0.0</td>
<td>61.9 ± 11.7**</td>
</tr>
</tbody>
</table>

Baseline and immediate post WAS VMR of mice non injected or injected sc with saline or naloxone (1 mg/kg) expressed in %. *p<0.05, **p<0.01 and ***p<0.001 vs respective baseline.

3. Pregnancy-related Changes in GABAAR δ Subunit Expression in Interneurons and Effects on Hippocampal CA3 Gamma Oscillations

I Ferando*, I Mody

UCLA Departments of Neurology and Physiology, *UCLA Molecular Cellular and Integrative Physiology Graduate Program

Neuroactive steroids (NS), such as allopregnanolone (ALLO) and THDOC, modulate the expression of extrasynaptic δ subunit-containing GABA Rs (δ−GABA Rs). δ−GABA Rs constitute a major class of GABA Rs that mediate tonic inhibition in the central nervous system. The δ subunit confers a high sensitivity to NS, which act on them as positive allosteric modulators. Acute stress, the estrus cycle and pregnancy are physiological conditions characterized by large alterations in NS levels, which are followed by brain-region-specific homeostatic changes in δ subunit expression. So far these changes have been described in principal cells, where δ−GABA Rs are typically paired with two α4 and two β subunits, while interneuronal δ−GABA Rs are predominantly paired two α1 and two β subunits.

Given its GABA Rs subunit distribution and characteristic physiology, the hippocampal CA3 region offers a unique opportunity to study interneuronal δ−GABA Rs. The tonic current of CA3 pyramidal cells is mediated by α5 containing GABA A Rs, while in this area δ−GABA Rs are expressed exclusively in interneurons. Gamma oscillations (35-90 Hz) are a rhythmic cortical network activity found in vivo and consistently reproducible in vitro. Gamma rhythms correlate with memory formation and retrieval, cognition, and sensory encoding. CA3 gamma oscillations are locally generated, and represent the expression of a synchronized
and finely regulated feedback loop of excitation and inhibition between local parvalbumin (PV) containing interneurons and CA3 pyramidal cells. Interneuronal tonic inhibition levels modulate interneuronal NMDA-R activity, thus controlling the frequency of CA3 gamma oscillations.

Here we demonstrate that interneuronal δ−GABA_A Rs are downregulated during pregnancy (murine day 18 of pregnancy, comparable to human 3rd trimester). We report for the first time that δ−GABA_A R expression can be independent of α4 subunits. Within 48 hours post-partum, interneuronal δ−GABA_A R expression is rapidly brought back to pre-pregnancy levels. The immunohistochemical findings were validated by an increase in 50 nM kainate-induced CA3 gamma oscillations frequency that was sensitive to the NMDA receptor antagonist DAP-5. These findings are similar to the changes found in virgin Gabrd-/- females. Post-partum, gamma frequencies of WT females revert to the lower frequencies found in virgin animals. However, mimicking the hormonal conditions during pregnancy by preincubating and recording slices of pregnant females in 100 nM ALLO, reduces gamma oscillation frequencies in slices of pregnant females to frequencies found in virgin mice.

Our findings indicate the importance of homeostatic changes in the brain during pregnancy to accommodate the large changes in neurosteroid levels. Disruptions or perturbations of this physiological mechanism, might lead to behavioral changes, temporary memory loss or reduced memory formation, and could bring about changes in neuronal excitability also in other areas of the brain in which δ−GABA_A R expression is altered in interneurons. Inaccurate coupling of NS levels to δ−GABA_A R expression could be responsible for abnormal psychiatric conditions that occur in response to stress or across the estrus-cycle and pregnancy, thus providing insights into potential new treatments.


Funding: This work was supported by NIH RO1 MH076994

4. Identification of eQTL Involved in Response to Behavioral Stressor – ANXA11 and TEF3

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¹UCLA Center for Neurobehavioral Genetics, ²University of Trier, Germany, ³Department of Neurology, UCLA, ⁴Wake Forest Univ. Health Sciences, Winston-Salem, ⁵Department of Human Genetics, McGill University, Canada ⁶Genome Sequencing Center, Washington University School of Medicine, St Louis, ⁷Department of Neurology, UCLA, ⁸Department of Psychiatry, UCLA

Genetic regulation of stress response is difficult to study in human populations due to variation in environmental conditions and stressor itself. To overcome these problems we investigated stress response under controlled condition in non-human primate (NHP) model, in the Vervet Research Colony (UCLA-WFU) which is an extended pedigree of African green monkeys also called vervets (Chlorocebus aethiops sabaeus). All colony animals were exposed to the same relocation stressor when the whole colony was moved from UCLA (low stress environment) to WF (new/unknown and therefore high stress environment). We took this opportunity to investigate gene expression levels in peripheral blood before and after the relocation stressor in the VRC memebers. To genetically map genetic loci regulating gene expression in stress related fashion (eQTL, expression Quantitative Trait Loci) we conducted genome wide microsatellite genotyping and gene expression assays (Ref-8v2 chip, Illumina) in 271 VRC vervet monkeys. We analyzed linkage between changes in gene expression levels using SOLAR and identified two loci, ANXA11 and TFE3, demonstrating cis and trans regulation, respectively. TFE3 (transcription factor E3) codes for a broadly expressed transcription factor which is a trans-activator of metabolic such as diabetes related genes IRS2 and HK2. ANXA11 (Annexin A11) codes for a protein which is a member of the annexin family, a group of calcium-
dependent phospholipid-binding proteins. ANXA11 is a susceptibility locus for sarcoidosis (a complex chronic inflammatory disorder).

To interpret the role of stress eQTL identified in blood, we performed transcriptome profiling (RNA sequencing) in blood, adrenal and pituitary tissues in 12 pedigree animals. Two stress eQTL transcripts identified in blood, ANXA11 (cis) and TFE3 (trans), showed correlation of expression level with adrenal tissue (0.64 and 0.58, respectively) indicating that these eQTL may also act in adrenal glands. We identified gene modules co-expressed with the eQTL using WGCNA (weighted gene coexpression network analysis). ANXA11 is a member of the gene module upregulated in adrenal tissue and significantly associated with GO terms related to mitochondrial functions.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Heritability</th>
<th>Heritability p-value</th>
<th>Max LOD</th>
<th>Marker</th>
<th>Cis/Trans</th>
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<td>TFE3</td>
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<td>1.33E-04</td>
<td>3.19</td>
<td>D10S186</td>
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</table>

5. Chronic Stress Increases Systemic and Fat Depot Cytokine Levels and Induces Inflammation-associated Signaling Cascades in Adipocytes

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Background and Aims: The importance of chronic psychological stress in the development of pathological conditions is well established. Fat tissue is among the organs significantly affected by stress. Given the central role of intra-abdominal fat depots in the development of metabolic disorders, the potential for stress to affect adiposity-associated diseases such as insulin resistance and type II diabetes is evident. We have previously shown dramatic effects of chronic unpredictable stress (CUS, a model of depression) on rat intra-abdominal adiposity and adipocyte responses to insulin stimulation. Here we studied the molecular mechanisms underlying these responses.

Methods: Rats were subjected to CUS (2 random stressors/d) for 35d and at the end of stress exposure, adipocytes were isolated from epididymal and mesenteric adipose tissue along with serum. RNA from adipocytes was subjected to microarray analysis for differential mRNA expression after CUS. Protein from adipocytes and serum were subjected to multiplex analysis to measure intracellular kinase activation and circulating cytokines, respectively.

Results: Stress induced a shift in adipocyte size within mesenteric and epididymal fat depots. Specifically, increased numbers of smaller adipocytes were observed in both depots. These changes were associated with increased circulating non-esterified free fatty acids (NEFA) levels (by 1.5-fold, p<0.01). CUS also increased levels of several cytokines, including TNFα, IL-6 and IL-1β, in both the serum and mesenteric fat. Adipocytes from stressed rats demonstrated differentially expressed mRNA transcripts for TNFα (p<0.01), IL-6 (p<0.05) and IL-1β (p<0.05). Using CUS-derived isolated adipocytes we found significant changes in the activation of intracellular signaling cascades involved in the inhibition of insulin signaling and the promotion of insulin resistance, such as JNK (p<0.01).

Conclusions: CUS induces activation of intracellular cascades involved in the inhibition of insulin signaling in adipocytes. These changes may be due to stress-induced changes in inflammatory cytokine profiles that we observe in the fat depots as well as the circulation in our stress model. Coupled with the increases in the circulating levels of NEFA, the molecular changes described here are reminiscent of obesity-induced effects on insulin sensitivity and glucose metabolism.

Support: NIH RC1DK086150, 1P50 DK64539, and the Broad Medical Foundation (BMRP)
6. **Contrasting Effects of Chronic Intermittent Ethanol Treatment on Membrane Properties and Synaptic Transmission in the Nucleus Accumbens and the Paraventricular Nucleus**

**V Marty** and I Spigelman

*Division of Oral Biology & Medicine, School of Dentistry, University of California at Los Angeles*

Alcohol withdrawal syndrome is a severe manifestation of alcohol abuse, presenting with a variety of symptoms such as anxiety and seizures. In this study we investigated the long-lasting effects of chronic intermittent alcohol treatment and withdrawal in two brain structures, the nucleus accumbens (NAcc) which plays a key role in drug-induced neuroadaptations underlying addiction, and the paraventricular nucleus of the hypothalamus (PVN) involved in the regulation of neurophysiological response to stress which plays a critical role in stress-induced alcohol relapse. We used a rat model of chronic intermittent ethanol (CIE) treatment (≥12 doses, 2.5-3 g/kg) followed by >40 days of withdrawal, which displays alterations in GABA<sub>A</sub>R subunits expression, function and pharmacological properties. Electrophysiological recordings were obtained using whole-cell patch clamp technique from coronal brain slices of CIE- or vehicle (CIV)-treated rats. Whole-cell patch recordings from medium spiny neurons (MSNs) in the NAcc core showed that the inward rectification induced by hyperpolarizing current pulses and the amplitude of the fast component of the action potential afterhyperpolarization were increased in CIE-treated rats. These CIE-induced modifications of electrical membrane properties could be due to an alteration in potassium-conductances. In addition, voltage-clamp recordings showed an increase in amplitude of AMPAR-mediated miniature excitatory synaptic currents (mEPSCs) in CIE-treated rats without modification in mEPSC frequency suggesting that CIE-induced facilitation of glutamatergic transmission is likely mediated by a postsynaptic mechanism. This CIE-induced plasticity likely modifies the integration and processing of inputs by MSNs in the NAcc leading to a lasting modulation of the brain reward system.

In contrast to the NAcc, we found that neither the amplitude nor the frequency of AMPAR-mediated sEPSCs were modified in the PVN of CIE rats. However, we found that the AMPA/NMDA ratio was significantly decreased in CIE-treated rats and was accompanied by an increase in NMDAR-mediated sEPSC amplitude but not frequency suggesting a postsynaptic mechanism. This CIE-induced plasticity in the PVN could induce a dysregulation in the neuroendocrine response to stress likely responsible for triggering relapse. Together this study revealed differential effects of CIE treatment on two important brain structures providing new understanding of the mechanisms involved in alcohol withdrawal and dependence.

**Funding:** NIH grant AA016100

7. **BDNF Triggers the Activation by Src Family Kinase Phosphorylation of the NMDA Receptors That Induce Substance P Release from Primary Afferents**

**W Chen, JA McRoberts, JCG Marvizón**

*Department of Medicine, UCLA; Veteran Affairs Greater Los Angeles Healthcare System*

There is ample evidence that primary afferent neurons express NMDA receptors, but their function is unclear. In particular, NMDA receptors in primary afferent terminals induce substance P release into the spinal cord (measured as NK1 receptor internalization), as shown by our findings in spinal cord slices and by one study in vivo (Liu et al., 1997, Nature 386:721). However, another group (Nazarian et al., 2007, Neuroscience 152:119) could not replicate these results in vivo. In fact, our own results in vivo agreed with those of Nazarian et al.: we found that intrathecal injections of NMDA plus D-serine (10 nmol each) did not induce NK1R internalization. Since these NMDA receptors are not functional unless they are phosphorylated by a Src family kinase (SFK) (Chen et al., 2010, Neuroscience 166:924), we hypothesized that they are in a dephosphorylated, non-functional state in normal conditions, and that they are brought out of it by signals upstream of the SFK. One of such signals could be BDNF. Indeed, we found that intrathecal BDNF enabled a subsequent (60 min later) intrathecal injection of NMDA to induce NK1 receptor internalization. The effect of BDNF was eliminated by the SFK inhibitors PP2 and dasatinib. The effect of intrathecal BDNF was potent,
dose-dependent and long-lasting. BDNF is released from dorsal horn microglia in some chronic pain conditions (Coull et al., 2005, Nature 438:1017); hence, we hypothesize that chronic pain may put these NMDA receptors in their functional state. To test this hypothesis, we injected NMDA intrathecally at different times after inducing chronic constriction injury (CCI) of the sciatic nerve in rats, a model of neuropathic pain. Intrathecal NMDA, but not vehicle, did induce NK1 receptor internalization after CCI. The effect of NMDA was maximal 6 hr after CCI and was present for as long as 3 days. It was blocked by an inhibitor of microglia activation (minocycline), a BDNF scavenger (trkB-Fc) and a SFK inhibitor (PP2). We concluded that after nerve injury BDNF is released by spinal cord microglia and activates trkB receptors in primary afferent terminals, leading to the SFK phosphorylation of these NMDA receptors, making them functional. These NMDA receptors probably participate in central and peripheral sensitization, leading to chronic pain.

8. Tissue-selective Knockout of NMDA Receptors in Nociceptive Afferents Abolishes Peripheral Sensitization to NMDA Receptor Agonists

JA McRoberts, S Jagannathan, HS Ennes, JCG Marvizón, EA Mayer, M Fanselow, JN Wood, B Vissel

Center for Neurobiology of Stress, Departments of Medicine & Psychology, David Geffen School of Medicine at UCLA; Wolfson Institute for Biomedical Research, University College London, London, UK; Neurobiology Research Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia

Peripheral injection of glutamate and other selective agonists of ionotropic glutamate receptors cause mechanical hyperalgesia in both humans and rodents. Since NMDA and AMPA receptors are expressed on peripheral afferent nerve terminals, it has been long assumed that this sensitizing effect was due to direct action of the glutamate analogs on the terminals. However, the recent discovery of NMDA receptors on non-neuronal tissues including lymphocytes and keratinocytes has made this assumption questionable. The aim of this study was to prove that NMDA receptors expressed on primary afferent terminals are indeed those responsible for the sensitizing effect of peripherally injected selective agonists. To this end, mice have been developed with tissue-specific knockout of NMDA receptors in a subset of nociceptive peripheral afferent nerves using Cre recombinase driven by the tetrodotoxin-resistant sodium channel Nav1.8 promoter and a floxed gene for the essential subunit of the NMDA receptor, NR1. These mice (Nav1.8-fNR1) have no obvious abnormalities and do not show any differences compared to wild type (Cre minus) littermates in response latencies to the hotplate (52.5°C) test, tail withdrawal from hot (48°C) water, or basal paw withdrawal responses to mechanical stimulation. Injection of 20 µl of a 1 mM NMDA solution into the footpad of the hind paw caused a 28 ± 5% decrease in paw withdrawal response 30 min later in Cre minus wild type mice, however this sensitizing effect was reduced to 6 ± 5% (P < 0.02) in Cre positive knockout mice. We observed a similar effect for 0.1 mM trans-ACBD, a higher potency NMDA receptor agonist, with a 23 ± 3% decrease in the control mice, but only a 2 ± 2% (P<0.002) decrease in the knockout mice. We also tested another strain of mice that has previously been described (Neurosci. 172:474, 2011) where the NMDA receptor is knocked down in all peripheral afferent neurons. These mice showed a similar reduction in paw withdrawal responses to trans-ACBD (from 46 ± 6% in Cre minus to 10 ± 9% in Cre+ mice; P<0.005). We conclude that NMDA receptors expressed on primary afferent terminals in the periphery mediate the sensitizing effects of glutamate analogues.

Supported by NIH grants DK058173 and 1 P50 DK064539

9. Increases in Voluntary Alcohol Consumption and Preference in a Rat Model of Post-traumatic Stress Disorder

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Post-traumatic stress disorder (PTSD) may develop after traumatic stressful events. PTSD patients exhibit high alcohol abuse rates. A rodent model of stress-enhanced fear learning (SEFL) mimics several of the
major features of human PTSD, including resistance to exposure therapy and amnesic treatment. The 2-bottle choice, intermittent access to 20% ethanol (EtOH) drinking paradigm leads to escalated voluntary EtOH intake. We used this paradigm to compare EtOH consumption in SEFL rats (n=10) and their conditioned controls (CTL; n=12). SEFL rats increased their EtOH consumption from 1.9±0.6 (1st presentation) to 6.3±0.7g/kg/24h (31st presentation). By contrast, CTL rats did not escalate drinking as quickly or to the same extent as SEFL rats (1.3±0.6 to 3.6±0.5 g/kg/24h). After 31 presentations, EtOH was withdrawn for 40 days and then reinstated. Upon reinstatement SEFL rats showed significantly higher EtOH consumption compared to CTL rats (p<0.05, 2-way RM ANOVA). To determine if SEFL rats increased EtOH consumption for its pharmacological effects, rats were given intermittent access to the 2-bottle-choice of water or 28.4% sucrose (caloric control for 20% EtOH), which also contained 0.08% quinine-HCl (S/Q solution) to control for EtOH’s aversive effects. Over 31 presentations, SEFL rats escalated S/Q solution consumption (1.0±0.3 to 9.1±2.6 g/kg/24h) similar to CTL rats (0.6±0.1 to 10.7±2.8 g/kg/24h). Two-way RM ANOVA revealed no group differences in QS solution consumption. These data suggest that the higher EtOH consumption by SEFL rats is due to the pharmacological effects of EtOH.

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10. Acute Water Avoidance Stress Induces Visceral Analgesia When Tested Non-invasively – Differential Role of Opioid-dependent Mechanisms in Male and Female Rats

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Background: Previous studies in male rats using electromyography to assess the visceromotor response (VMR) to colorectal distension (CRD) showed a delayed visceral hyperalgesia 24h after an acute (1 day) or repeated (10 days) exposure to water avoidance stress (WAS). Recently, we reported that rats tested non-invasively exhibit a robust immediate analgesia in response to repeated WAS.

Aims: To assess non-invasively in rats the effects of acute WAS on the VMR to CRD, the role of opioids in stress-related pain modulation and whether there are sex differences.

Methods: Male (M) and female (F) Wistar rats (7-8 weeks, 2-4/cage) were monitored for VMR to CRD (10, 20, 40, 60 mmHg, 20 sec duration, 4 min intervals) using a novel manometric technique. The 1st CRD (day 0) served as baseline, then rats were exposed to 1h WAS. One group (F11, M10) received no injection. Two other groups were injected subcutaneously with naloxone (1 mg/kg) (F10, M9) or saline (0.3 ml) (F12, M12) 10 min before WAS. The 2nd CRD was monitored 45-50 min after WAS (day 1) and the 3rd CRD, 24 h after WAS (day 2). The VMR after WAS was expressed as percentage of the respective baseline values at different CRD pressures. Data were analyzed using 2-way ANOVA and Bonferroni post-hoc test.

Results: On day 1, both females and males exhibited a visceral analgesic response present at 40 and 60 mmHg (VMR: 52.9±11.5% vs 111.8±24.6%, p<0.001 and 69.1± 1.1% vs 100.0±0.0%, p<0.05) in females and only 40 mmHg (VMR: 47.6±8.6% vs 88.6±10.7 %, p<0.001) in males. On day 2, 24 h after WAS, the VMR was not different from baseline values in females and males. Saline-injected females exhibited visceral analgesia at 60 mmHg on day 1 and hyperalgesia at 40 and 60 mmHg on day 2 (p<0.05), which were both prevented by naloxone pretreatment. In contrast, saline or naloxone-injected males did not show significant changes in VMR compared to baseline neither on day 1 nor 2.

Conclusions: When monitored non-invasively, acute WAS induces visceral analgesia in both female and male rats. Subcutaneous injection as an additional noxious stress stimulus alters visceral sensitivity to CRD in a time and sex-dependent manner. Only in females, opioid-dependent mechanisms participate in the modulation of immediate visceral analgesia and delayed visceral hyperalgesia.

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11. Blast-type Traumatic Brain Injury Alters Intestinal Contractility and Barrier Integrity in Rats

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**Background:** The effects of blast-type traumatic brain injury (bTBI), a common combat-related medical problem, are not exclusive to the area of injury but also to air-filled soft tissues. The gastrointestinal tract (GIT) has bidirectional interaction with the brain and is an air-filled organ making it susceptible to bTBI. It is unclear how bTBI affects the GIT.

**Aim:** To determine whether acute and chronic bTBI result in the impairment of the GIT contractility and the modulation of intestinal tight junction proteins, intestinal cytokines and CRF receptor expressions.

**Methods:** Anesthetized male SD rats were exposed to either a single acute dTBI (air blast, 172-206 kPa, <1 sec) or to chronic bTBI where rats were exposed to three separate blasts in two weeks intervals. Rats were euthanized 24 hours after the last dTBI. Intestinal sections were analyzed for protein and RNA expression of cytokines, tight junction proteins, CRF receptors and mounted on slides for IHC. The area under the curve (AUC) of colonic contractions was monitored using non-invasive manometry.

**Results:** Cytokine array profile of acute bTBI revealed proinflammatory IL-6 and TNF-α increases of 2.2-fold and 1.9-fold in ileal tissue and 3.5-fold and 2.5-fold in colonic tissue, respectively. PCR profile showed an increase in ileal occludin, ZO-1 and claudin-2 expression in acute bTBI compared to sham, while claudin-1 was decreased. CRF receptor type 2A (CRF2a) was increased in acute bTBI compared to sham but differences in type CRF2b receptor were not significant. Ileal IL-6, TNF-α, occludin, ZO-1 and claudin-2 RNA expressions were increased in chronic bTBI compared to sham, while in contrast claudin-1 was increased. Acute bTBI decreased AUC of proximal colon contraction (39.6±6.0 vs 19.1±3.1 in sham, p<0.05) while contraction in chronic bTBI tended to increase (104.6±38.1 vs 29.3±5.0 in sham, p>0.05).

**Conclusion:** bTBI injury affects the GI tract by impairing colon contractility, altering mucosal barrier integrity. bTBI also up-regulates intestinal CRF2a receptors which may play a role in the intestinal CRF/urocortin signaling. Thus changes in these GI functions are useful indicators of the severity of injury following acute and chronic bTBI.

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12. Mice Over-expressing Wild-type Human Alpha-synuclein Display Alterations in Colonic Myenteric Ganglia and Propulsive Colonic Motor Function

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**Background:** Gastrointestinal (GI) symptoms are one of the most prevalent non-motor symptoms of Parkinson’s disease (PD), and significantly impact patients’ quality of life. Despite some recent progress, there remains a paucity of suitable animal models to study the mechanisms of PD-related GI symptoms and test new treatments. Alpha-synuclein, a protein associated with both familial and sporadic PD forms pathological neuronal aggregates in PD, including in myenteric neurons. Therefore, mice overexpressing α-synuclein may provide a suitable model for assessing PD-related GI deficits. We have previously shown colonic dysmotility in 12 months old mice overexpressing human wild type α-synuclein under the Thy1 promoter (Thy1-aSyn).

**Aim:** To investigate gut alterations in Thy1-aSyn mice at earlier ages as well as expression of α-synuclein and its relationship to other neurotransmitters in the distal colon.

**Methods:** Defecation, gastric emptying (GE) and immunoreactivity of α-synuclein, peripheral choline acetyltransferase (pChAT), tyrosine hydroxylase (TH), neuronal nitric oxide synthase (nNOS) and vasoactive

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intestinal peptide (VIP) in distal colon myenteric plexi were assessed in Thy1-aSyn mice.

Results: Thy1-aSyn mice aged 2.5-3 or 7-8 months old had 81% and 55% reduction in fecal pellet output, respectively in response to the first 15 min of 2 h in novelty, and 60% and 69% reduction, respectively during the subsequent 1-h refeeding compared with age matched WT littermates. The reduction of fecal pellets was not correlated to food intake during the 1 h of refeeding ($r^2 = 0.03$, $p > 0.05$). In the early dark phase 8 months old Thy1-aSyn mice showed a 59% reduction of fecal pellets also occurred in the first 15 min of novel environment. Thy1-aSyn mice (8-10 months) displayed increased α-synuclein in myenteric plexi with abundant varicose terminals surrounding pChAT-immunoreactive (ir) neurons and only a few, nNOS-ir neurons. There were no conspicuous changes in pChAT- and nNOS-ir neurons, and TH- and VIP-ir nerve fibers, or proteinase K-resistant α-synuclein aggregates at the ages examined. GE was not altered in Thy1-aSyn mice aged 4 to 18 months.

Conclusions: The occurrence of colonic dysfunction in Thy1-aSyn mice several months before the loss of striatal dopamine together with the histological data support the hypothesis that over-production of presynaptic α-synuclein in colonic myenteric ganglia could interfere with cholinergic neuronal activation, causing early functional bowel symptoms in PD.

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13.

A Novel Neuropeptide, Teneurin C-terminal Associated Peptide-1 (TCAP-1) Is Expressed in Colonic Enteric Neurons and Potentiates Corticotropin-releasing Factor (CRF)-induced Defecation in Rats

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Background: Central or peripheral CRF evokes stress-like responses by stimulating colonic motility and inducing visceral hyperalgesia in rodents (Taché et al., 2009). TCAP-1, a novel neuropeptide found in the rat brain shares 20% sequence homology with CRF while not binding to CRF receptors. Repeated injections of TCAP-1 either enhance or attenuate central CRF-induced alterations in behaviors and neuronal activities via actions downstream to CRF receptors (Lovejoy et al., 2009).

Aims: In rats, 1. To map TCAP-1 gene expression in the GI tract. 2. To identify the cellular localization of TCAP-1 in the colon. 3. To characterize the effects of repeated subcutaneous (sc) injections of TCAP-1 on intraperitoneal (ip) CRF-induced defecation and visceral hyperalgesia.

Methods: Esophagus (E), foregut (F), gastric corpus (GC), antrum (A), duodenum (D), jejunum (J), ileum (I), cecum (C), proximal and distal colon (pC and dC) and rectum (R) were collected from 2 SD naïve male rats. TCAP-1 gene expression was detected by RT-PCR. Colonic paraffin tissue sections and whole mount preparations of the submucosal and myenteric plexus were processed for immunohistochemistry using antibodies raised against C- or N-terminal region (CT or NT) of rat TCAP-1. Adult male SD rats (8-11/group) received once daily sc injection of TCAP-1 (1000 pmol/kg) or saline (0.3 ml) for 10 days, followed by ip injection of CRF (1.5, 3 or 10 µg/kg) or saline (0.3 ml). Fecal pellet output (FPO) and diarrhea score (DS) were monitored and visceromotor response (VMR) to noxious colorectal distension (CRD) was assessed by solid-state manometry (Larauche et al., 2009) for 1h post ip injection.

Results: TCAP-1 transcript was detected in all GI segments except for the E with highest levels in the GC, A, D, J and pC. There was a 2.2-fold higher level in the pC than dC. The immunoreactivity for TCAP-1 was localized in the submucosal and myenteric neurons with strong labeling in both soma and processes by CT antibody and only in soma by NT antibody. In Thy1-aSyn treated rats, the subthreshold dose of CRF (1.5 µg/kg) increased FPO/1h (TCAP-1/CRF: 4.5±1.5 vs vehicle/vehicle: 0.0±0.0, $p<0.01$) and DS (TCAP-1/CRF: 1.8±0.4 vs vehicle/vehicle: 0.0±0.0, $p<0.001$, or vs vehicle/CRF: 0.7±0.2, $p<0.01$) while either CRF or TCAP-1 alone did not induce a significant increase in FPO/1h and DS. IBS-like symptoms such as diarrhea,
defecation and hypersensitivity induced by ip CRF at other doses were not affected by TCAP-1 compared to vehicle.

**Conclusions:** TCAP-1 is widely distributed in rat GI tract and localized in colonic enteric neurons where it potentiates secretory motor function of CRF given at a subthreshold dose suggesting an interaction between these peptides that may have implications in colonic response to stress.

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14.

**Cardioprotection of Electroacupuncture against Myocardial Ischemia-Reperfusion Injury by Modulation of Cardiac Sympathetic Nerve Activity**

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Augmentation of cardiac sympathetic tone during myocardial ischemia has been shown to increase myocardial oxygen demand and infarct size as well as induce arrhythmias. Such effects can lead to further degradation of myocardial performance and worsen the outcomes of a heart attack. Myocardial ischemia is associated with a marked increase of norepinephrine (NE) in ischemic tissue. In-vivo measurement of NE has been used as a surrogate marker of sympathetic nerve activity. We previously demonstrated that electroacupuncture (EA) inhibits the visceral reflex-induced hypertension. The purpose of this study was to determine the effects of EA on left ventricular (LV) function, oxygen demand, infarct size, arrhythmogenesis, and cardiac sympathetic nerve activity by measuring in-vivo cardiac NE concentrations in a myocardial ischemia-reperfusion (MIR) model. Anesthetized rabbits (n = 30) underwent 30 min of left coronary artery occlusion followed by 90 min of reperfusion. We evaluated myocardial oxygen demand, infarct size, ventricular arrhythmias, and myocardial NE release using microdialysis, under the following experimental conditions: 1) untreated; 2) EA at P5-6 acupoints; 3) sham acupuncture; 4) EANal, EA with pretreatment with naloxone (non-selective opioid receptor antagonist); 5) EANal+Che, EA with pretreatment with naloxone and chelerythrine (non-selective protein kinase C inhibitor). Compared to untreated and sham acupuncture groups, EA resulted in decreased oxygen demand, myocardial NE concentration, and infarct size. Furthermore, the degree of ST segment elevation and severity of LV dysfunction and ventricular arrhythmias were all significantly decreased (P<0.05). The cardioprotective effects of EA were partially blocked by pretreatment with naloxone and completely blocked by pretreatment of both naloxone and chelerythrine. In conclusion, EA at S-6 decreased MIR-induced ventricular dysfunction, infarct size, occurrence of arrhythmias and elevated-interstitial NE level, through opioid and PKC-dependent mechanisms. Our data suggest that EA produces cardioprotective effects against MIR through an inhibition of the cardiac sympathetic nervous system and may play an important role in clinical treatment of cardiac ischemia.

**Figure 1. Interstitial norepinephrine levels before and during myocardial ischemia and reperfusion.** * P < 0.05 as compared with the baseline. † P < 0.05 EA vs. control group.
Abstracts of Posters
Clinical

15. Autonomic Dysregulation in Response to a Visceral Stressor in Irritable Bowel Syndrome

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Background: Irritable bowel syndrome (IBS) is a functional GI disorder characterized by abdominal pain with diarrhea and/or constipation. Recent studies suggest that autonomic dysregulation may play a pathophysiologic role in IBS. We examine autonomic nervous system (ANS) responses at rest and during a visceral stressor in IBS patients and healthy controls (HCs) to explore autonomic dysregulation in IBS. Furthermore we examine the effects of sex, anxiety, depression, current IBS symptom ratings, and perception of visceral stressor on ANS function.

Aims: 1) To compare heart rate variability (HRV) and skin conductance (SC) at rest and during a visceral stressor in IBS patients and HCs and 2) To examine the effects of sex, psychological symptoms and perceptual ratings of the visceral stressor on ANS function.

Methods: Male and female IBS patients and HCs aged 18-55 underwent a structured psychiatric interview (MINI) and completed the Hospital Anxiety and Depression Scale, Bowel Symptom Questionnaire, and Verbal Descriptor Visual Analog Scale. EKG and SC measurements at rest and during a visceral stressor were acquired for heart rate variability autoregression analysis. Linear mixed models using the SAS v9.2 Mixed procedure were performed as well as the Wilcoxon Rank Sum test, Fisher Exact test, and Spearman correlation. Significance was determined as having p < 0.05.

Results: Data from 31 IBS patients (58.1% female) and 26 controls (65.4% female) were used. IBS patients showed significantly lower cardiovagal tone at rest compared to controls (p = 0.0356) and showed less overall response in ANS activity during the study protocol. Males had significantly higher cardiosympathetic tone than females (p = 0.002) but no group differences were found within each sex. Sympathetic tone positively correlated with perception of unpleasantness during the visceral stressor in IBS (r = 0.592, p <0.001) but not in controls.

Conclusion: IBS is associated with autonomic dysregulation with IBS patients having a lower cardiovagal tone at rest but a lack of response to visceral stressor and to the effect of anxiety. In IBS patients, autonomic arousal during the visceral stressor was associated with their perceived unpleasantness during the procedure, suggesting that sympathetic nervous system activity may be an objective physiological correlate of visceral hypersensitivity in IBS.

16. Sex Differences in Emotional Reactivity in Irritable Bowel Syndrome (IBS) and Human Controls (HC)- an fMRI Study

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Background: Differences in the relative engagement of cortical and affective brain regions have been demonstrated between female and male IBS patients during rectal distension and during an abdominal pain expectation task. However, it is not known if such sex related differences are specific to abdominal pain related task, or if they are only seen in IBS patients.

Aims: To investigate sex differences and IBS specific differences in emotional-arousal circuitry during an emotional reactivity task, unrelated to IBS symptoms. Based on our previous work, we hypothesized greater...
brain responses to the task would be seen in brain networks related to prefrontal and to central autonomic control (insula cortex [INS], amygdala) in both IBS and HC males.

**Methods:** Brain response was measured in 72 female (38 HC, 34 IBS) and 46 male (26 HC, 20 IBS) subjects using a Siemens Allegra 3T MRI scanner. Subjects were asked to view and match negatively valenced faces (Face) or geometric forms (Form). The contrast between these two viewing conditions (Face-Form) is considered an index of “emotional reactivity”. Analyses were restricted to a homogenous group of individuals demonstrating emotional reactivity in the right amygdala at a liberal criteria of p<.20. Sex differences during emotional reactivity were tested in apriori specified regions comprising emotional-arousal, cortico-modulatory and homeostatic afferent circuitry by applying a second-level random effects general linear model controlling for subject and using an implicit baseline.

**Results:** 32 HC (7 male) and 27 IBS (10 males) who met criteria for emotional reactivity were included. In all subjects significant BOLD responses were observed in regions including anterior INS, amygdala, hippocampus, anterior mid cingulate cortex (aMCC), and dorsal and ventral lateral prefrontal cortices (PFC). Males, both IBS and HC, showed greater brain activity in response to the task. IBS males showed greater activation in INS subregions, PFC subregions (left dorsal medial PFC, left dorsal and ventral lateral PFC) and right amygdala compared to IBS females. HC Males showed greater response than HC females in the posterior INS, and the right hippocampus. No regions tested showed greater reactivity in female IBS or HC subjects.

**Discussion:** In this group of emotional responders, a GI symptom unrelated emotional reactivity task was associated with greater engagement of prefrontal modulatory regions and INS cortex in male subjects, regardless of disease group. These findings are consistent with previous findings using abdominal pain related tasks in both human subjects and rodents, and suggest a generalized sex difference in the response to the brain to a variety of emotionally salient stimuli.

### 17. Global Differences in Nuerokinin 1 Receptor Density in Patients with IBS and IBD

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Both irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) have similar symptom complexes that result in chronic abdominal discomfort and altered bowel habits. While IBD may be directly related to peripheral and central sensitization of visceral pain pathways, there is an absence of detectable pathology accounting for IBS symptoms. Data suggest differences in substance P (SP)/NK-1 signaling between IBS and IBD in animal models. However, little is know about this relationship in humans. Positron emission tomography (PET) with an NK-1 receptor ligand has recently shown that chronic pain patients have lower levels of receptor availability in brain regions crucial to pain processing when compared to controls. This suggests that chronic pain may be associated with a downregulation of supraspinal NK-1 receptor expression.

No study has assessed differences in NK-1 receptor expression in the brain of patients with IBD or IBS. The aim of this pilot study was to determine if patients with long-standing chronic pain from either IBD or IBS demonstrate reduced NK-1 receptor expression compared to controls, and to examine whether the pattern of expression differs between the two patient populations. Additionally we sought to determine the relationship between NK1-receptor expression and sensitivity to pain as measured by a painful thermal stimulus.

We used PET and an NK-1 receptor radioligand, [18F]SPA-RQ, to quantify NK-1 receptors in the brains of controls and patients with IBD and IBS. 9 subjects from each population underwent PET scanning followed by a series of thermal stimuli used to measure acute pain threshold. Whole brain binding potential (BP) maps were generated (PMOD and SPM8) to assess NK-1 receptor BP distribution. Results show that compared with controls, BP is lower in IBS and further reduced in IBD demonstrating different levels of expression in each population. In addition, BP was negatively correlated with sensitivity to pain in patients with IBS but not IBD. These preliminary results demonstrate widespread global differences between healthy controls and chronic pain patients, and differences in pain processing and NK-1 receptor expression between patients with
IBD and IBS. Further research is needed to better understand the mechanisms that link downregulation of NK-1 receptor expression among patients with IBD and IBS.

18. Beta-arrestins and Endothelin-Converting Enzyme-1 Activity Mediate Neurotensin-induced Pro-inflammatory Signaling in Human Colonocytes

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Background and Aims: Neurotensin (NT) receptor-1 (NTR1), the high affinity NT receptor, mediates NT-associated intestinal inflammatory and cell proliferative responses in vivo and in vitro. NT induces interleukin-8 (IL-8) expression and cell proliferation in NCM460 human colonic epithelial cells overexpressing NTR1 (NCM460-NTR1) through MAP kinase and NF-κB activation. Endothelin-converting enzyme-1 (ECE-1) is involved in G protein-coupled receptor (GPCR) recycling and cell re-sensitization, while beta arrestin-1 (βARR1) and beta arrestin-2 (βARR2) terminate G protein-dependent signaling and are involved in NTR1 internalization. The aim of this study was to examine the role of βARRs and ECE-1 in NT-induced pro-inflammatory responses in human colonic epithelial NCM460 cells.

Methods: Co-localization of NTR1, βARRs and ECE-1 in the early endosomes in NCM460-NTR1 cells was examined under confocal microscopy after NT exposure. HPLC and MALDI-TOF mass spectrometry was used to detect fragments from the degradation of NT by recombinant human ECE-1 under different pH conditions. ERK1/2 and NF-κB activation of NT-stimulated NCM460-NTR1 cells pre-treated with the ECE-1 inhibitor, SM19712, and si-RNA of βARR-1 and βARR-2 were examined by western blot and NF-κB-driven luciferase assay. Expression of pro-inflammatory cytokines was verified by ELISA and qPCR.

Results: βARR1 and βARR2 were translocated to ECE-1-containing early endosomes together with NTR1 after NT exposure. Recombinant ECE-1-degraded NT only in physiological pH of early endosomes (pH: 5.5). Pre-treatment of NCM460 cells with the ECE-1 inhibitor SM19712 and gene silencing of βARR2 attenuated NT-stimulated NF-κB activation and IL-8 secretion was reduced by ~60%. Significant reduction in TNF-α and IL-1β transcription was also observed (p<0.05). In addition, inhibition of ECE-1 activity and gene silencing of βARR1 significantly reduced NT-induced ERK1/2 and JNK phosphorylation. JNK phosphorylation was only involved in NT-induced cell proliferation, but not IL-8 transcription in response to NT.

Conclusions: βARRs and ECE-1 activity regulate NT-induced MAP kinase and NF-κB activation in human colonic epithelial cells that are associated with cell proliferation and secretion of proinflammatory cytokines. This novel NTR1-dependent pathway may be important in the pathophysiology of colitis regulated by NT.

19. Depression Attenuates Cerebral and Peripheral Vascular Responses to the Valsalva Maneuver in Obstructive Sleep Apnea

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Obstructive sleep apnea (OSA) patients show elevated depressive symptoms and cardiovascular dysfunction, possibly from neural injury in regions regulating those functions. Since depression predicts worsening cardiovascular outcomes in other conditions, depression in OSA may be associated with compromised peripheral and cerebral vascular function. We studied 47 recently-diagnosed, untreated, moderate-to-severe OSA patients (AHI = 33±21 events/hour; age = 44±9 years; female:male = 12:35), with no history of mental illness. We classified subjects with the Beck Depression Inventory-II (BDI) into “low” (BDI≤9; N=25), “mild” (10≤BDI≤19; N=7) and “mod-severe” (BDI>19; N=5) groups. We collected blood-oxygen level dependent (BOLD) magnetic resonance imaging and heart rate measurements while subjects performed a Valsalva
maneuver, an 18 second forcible exhalation against a closed glottis, which normally elicits substantial transient blood pressure and heart rate changes. Relative changes in cerebral blood flow were calculated from the global BOLD signal. Group-by-time differences in heart rate appeared, with the low group showing the greatest heart rate responses, followed by mild, and with mod-severe group the lowest (Fig., $P<0.05$, repeated measures ANOVA). The low group showed the greatest post-release overshoot (4 s post) and subsequent dip (11 s post), followed by the mild and mod-severe groups. BOLD responses were reduced in the mild and mod-severe groups, with the greatest impairment in the mod-severe group, at 3 s into the challenge, and after release at 1-4 s and 14-24 s. Depressive symptoms in OSA are accompanied by reduced cardiovascular and cerebral blood flow responses to an autonomic challenge. The impairments may result from tissue injury in insular and cingulate areas, which are damaged in OSA patients with depression. Depressed mood in OSA, as in other disorders, may worsen cardiovascular outcomes, and the reduced cerebral blood flow responsiveness may contribute to further brain injury in the condition.

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20.

Early-Life Stress Effects on Behavioral Persistence

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Early-life stress is associated with a variety of negative health outcomes, but the cognitive processes behind these outcomes are poorly understood. We examined the impact of early-life stress on the partial reinforcement extinction effect, the tendency to persist in performing a previously rewarded behavior under extinction following partial reinforcement. Participants were rewarded for entering correct button-press sequences on a continuous reinforcement schedule (100%) or a partial reinforcement schedule (50%). This acquisition phase was followed by a period of extinction during which no rewards were delivered. Multiple regression analysis revealed a three-way interaction effect of early-life stress, neuroticism, and reinforcement schedule on the number of goal responses entered in extinction. Simple effects tests indicated that the partial reinforcement extinction effect is reduced in people who are high on neuroticism and have experienced early-life stressors. This lack of behavioral persistence may contribute to the poor health outcomes observed in this population.
21. Evaluation of Therapeutic Yoga and Walking for Patients with Irritable Bowel Syndrome: Preliminary Findings

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Irritable Bowel Syndrome (IBS) is the most common of the chronic functional gastrointestinal disorders and is associated with alterations in brain-gut interactions. Based on limited success of traditional medical treatments, mind-body approaches have been proposed, and there is a growing literature on the effectiveness of cognitive behavioral approaches and a growing interest in movement-based therapies such as yoga. This study aimed to evaluate two movement-based therapeutic treatments for patients with IBS. The study sample included 18 to 65 year old adults with chronic abdominal pain or discomfort and associated changes in bowel habits. All subjects met Rome III criteria for IBS and identified IBS as their primary medical complaint. Participation involved random assignment into either an Iyengar yoga or a structured walking program. Each program included 16 group sessions, held twice a week for 8 weeks. The Iyengar yoga postures were specifically tailored for patients with abdominal pain and discomfort, and the walking program consisted of non-aerobic moderate-paced outdoor walking. Twenty-five subjects have to date completed treatment (15 in yoga and 10 in walking). Results for the Yoga-treated subjects revealed a significant decrease from pre to post treatment in IBS symptom severity (p=.001), gastrointestinal-symptom-specific anxiety (p<.05), trait anxiety (p<.05), and pain catastrophizing (p<.05). For subjects assigned to the walking treatment, significant decreases were found in general negative affect (p<.05), dysphoria (p<.05), trait anger (p<.05), and state anxiety (p<.05) as well as health worry (p<.05), and a significant increase in serenity (p<.05). Preliminary results from this study therefore suggest that movement-based treatments may be beneficial for patients with IBS but that the two treatments have somewhat differential effects. A targeted Iyengar yoga program may be more effective for IBS symptoms while a group walking program (perhaps due to the social interactions) may show most impact on general mood and health worry. Qualitative data will also be presented, and the full study will include increased subject numbers and 6-month follow-up data.

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22. Increased SP Proinflammatory Responses in Human Mesenteric Preadipocytes Isolated from Ulcerative Colitis Patients

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Background and Aims: The neuropeptide substance P (SP), via binding to its high affinity receptor (NK1R) is involved in the pathophysiology of Inflammatory Bowel Disease (IBD). We have previously shown that intracolonic administration of TNBS to mice is associated with inflammatory changes in the mesenteric fat depots, including increased expression of NK-1R. We also showed that SP has proliferative, anti-apoptotic, metabolic, and pro-inflammatory effects on human preadipocytes that may affect IBD pathophysiology. Here we compared the direct effects of SP on human mesenteric preadipocytes isolated from control subjects (C) and ulcerative colitis (UC) patients.

Methods: Human mesenteric adipose tissue was collected during operations from control (n=4), or UC (n=4) patients. Preadipocytes were isolated from the fat depots, cultured, and exposed to 10^{-7}M SP for either 8 hours or of 5, 10, 20, 30, and 60 minutes. RNA, supernatants and total protein were collected. mRNA expression of NK1R and the inflammatory cytokines IL-1β and IL-8 was determined with qPCR and the activation of p-38 MAPK and ERK1/2 with western blot.

Results: Human mesenteric preadipocytes from UC have higher basal mRNA levels of NK1R (>10-fold), compared to controls, although this response did not reach statistical significance. In addition, preadipocytes isolated from UC patients demonstrate increased proinflammatory cytokine mRNA expression in response to
SP treatment compared to those isolated from control subjects. In particular, we observed higher mRNA expression of both IL-1β and IL-8 (p<0.02 and p<0.03 respectively) after SP treatment in UC-derived mesenteric preadipocytes. Furthermore, SP induced activation of both p38MAPK and ERK1/2 in UC-derived preadipocytes, in contrast to control preadipocytes where activation of these kinases was either reduced (ERK) or unaffected (p38).

Conclusions: Our data suggest a distinct disease-dependent proinflammatory effect of SP on preadipocytes, which may be due to the different expression levels of NK1R and/or altered state of downstream regulatory pathways. Our preliminary evidence suggests a potential role for p38 MAPK and ERK1/2 signaling pathways in the regulation of differential cytokine responses between control and UC-derived preadipocytes.

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23. Abnormal Resting State Activity of the Brain in Irritable Bowel Syndrome

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Background and Aims: Alterations of brain structure and function have been reported in patients with persistent pain disorders, including irritable bowel syndrome (IBS). Resting state (RS) brain activity in IBS has not been described. We aim to identify alterations in RS brain activity in patients with IBS, compared to healthy control subjects.

Methods: Subjects with Rome III positive IBS and healthy control subjects underwent a 10 minute, eyes open, RS functional MRI. The IBS Severity Scoring System was used. Fractional amplitude of low frequency fluctuation (fALFF, using low frequency band .01-.08 HZ) analysis was performed with two sample t-tests determining group differences, using a threshold of p<.001 uncorrected, cluster size 10 for whole brain significance and p<.05 corrected by family-wise error rate for region of interest analyses in the insula. Correlations with symptom severity and duration were performed in SPM8. Seed based connectivity was used to assess group differences in the connectivity of an insula-based network.

Results: In two samples of IBS patients (n = 17 and n=9), fALFF was increased in the right anterior insula (aINS) of IBS patients compared to healthy controls (n=15 and n=10). Right aINS fALFF correlated with symptom severity (pFWE=0.04) and duration (pFWE=0.04). Seed based connectivity showed that the right aINS had increased connectivity with the thalamus, nucleus accumbens, anterior midcingulate cortex, dorsolateral prefrontal cortex, and amygdala in IBS compared to healthy control subjects.

Conclusions: Irritable bowel syndrome patients exhibit altered RS brain activity in the aINS, which correlates with symptom severity.

24. The Corticotropin Releasing Factor 1 Receptor (CRF1R) Antagonist GW876008 Modulates Brain Response during Extinction in Patients with Chronic Abdominal Pain, but Not Healthy Control Subjects

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Background: Engagement of the central CRF/CRF1 receptor signaling system is involved in the central coordination of the stress response, and in emotional learning. Alterations in this system have been implicated as a possible mechanism in pathophysiology of irritable bowel syndrome (IBS). Conditioned fear responses to abdominal pain and discomfort are likely to play a role in IBS symptoms. The aims of this study
were to characterize the effect of the CRF1 receptor antagonist on brain responses during acquisition and extinction of conditioned fear to an abdominal pain stimulus in age-matched female IBS patients (n = 11) compared to healthy controls (HC; n = 15) using a 2 group (IBS, HC) x 3 drug (placebo [PLA], 20 mg and 200 mg of GW876008) cross-over design, using functional magnetic resonance imaging.

**Experimental Paradigm:** The fear conditioning and extinction learning protocol consisted of three phases: 1) Acquisition [5 trials of cue presentation (red light) followed by an aversive abdominal stimulation (electric shock to left lower abdomen)]; 2) Test phase [10 trials in which stimulation followed cue on 50% of the trials; and 3) Extinction (5 trials presenting cue but no stimulation). Trial duration was 15 s as was each inter-trial interval. SPM8 was used to specify a general linear model with subject as a random effect and group and drug as factors. A priori contrasts were conducted to test for group differences in brain response to the cue during GW876008 compared to PLA administration. Anatomically defined region of interests using subject-level beta images representing mean blood oxygenation level dependent (BOLD) signal changes during the Acquisition or Extinction cluster were utilized. Voxel-level significance was interpreted at p<0.05 after implementing family-wise error correction.

**Results:** Within-group analyses indicated that the antagonist significantly suppressed clusters of activity in the thalamus and midbrain regions in both patients and HCs during Acquisition. Group differences were observed in the mPFC during Acquisition. During Extinction, the CRF1 receptor antagonist produced greater suppression of brain activity in IBS compared to HC for B medial prefrontal cortex (mPFC), B pons, right hippocampus (Fig 3B) and L anterior Insula (aINS). Within group analyses indicated that for IBS patients, 200 mg of the antagonist produced suppression bilaterally in the mPFC, midbrain, hippocampus, thalamus, aINS and right pons where as in HCs significant suppression was only observed in the L aINS.

**Conclusions:** CRF1 receptors appear to play a similar role in fear acquisition in both IBS patients and healthy controls. However, CRF1 receptors may play a greater role in IBS as compared to HCs during extinction most notably in regions known to modulate extinction of fear (mPFC & hippocampus).

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25. **Puberty and Sex Differences in Diffuse Noxious Inhibitory Controls among Youth**

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Diffuse Noxious Inhibitory Controls (DNIC) involves the activation of endogenous analgesia via descending pain-modulatory systems. The DNIC effect of “pain inhibiting pain” has been widely demonstrated in adults. Yet, no extant work has examined DNIC among youth, nor has prior research tested sex or puberty effects on DNIC in this population. Ninety-nine youth (53 girls), aged 8-17 years (mean=13.5) completed a test of DNIC which measured pain ratings for a test stimulus (TS; 5 sec pressure to the left thumbnail) when it was administered: alone (TS1); during a conditioning pain stimulus (CS) (30 sec cold water immersion of the right hand) (TS2); 15 sec after CS termination (TS3) and 50 sec later (TS4). Pain ratings using a 0-10 Numerical Rating Scale (NRS) were made immediately following each TS administration. Pubertal status was assessed via self-reported Tanner stage and categorized as early (Stages I and II; n = 28) vs. late puberty (Stages III-IV; n = 71). Two-way between (Sex)-within (Time) repeated measures ANCOVAs with age as a covariate were conducted on the 4 TS ratings in the early puberty (EP) and late puberty (LP) groups separately. In the EP group, there were no main effects of Time or Sex, and no significant interaction effects, although age was a significant covariate. In the LP group, there was a significant main effect of Time [F(3,66)=23.35, p< .001], but no main effect of Sex, nor any significant interaction effects; age was not a significant covariate. Pairwise comparisons indicated that pain ratings for TS2 and TS3 were significantly lower than TS1 and TS4; TS2 was lower than TS3, but TS1 and TS4 did not differ. These results suggest a significant DNIC effect during exposure to the CS which persists, albeit in an attenuated form, for a brief period after termination of the CS, but which dissipates following a longer recovery period. Notably, this DNIC effect was only evident among LP youth and was not associated with age. Older age was associated with lower pain sensitivity overall among EP youth. Thus, in this sample of non-clinical youth, there is a measurable DNIC response that is present following the onset of puberty and this developmental change in the descending endogenous analgesia system is not accounted for by age alone. Further work is needed to elucidate the mechanism by which puberty exerts this effect.
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