



Sixth Annual

**Basic and Translational
Science Symposium**

of the

**UCLA Center for
Neurobiology of Stress**

January 29, 2009

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Program

CENTER for NEUROBIOLOGY of STRESS

In collaboration with the UCLA Brain Research Institute, the UCLA Semel Institute for Behavioral Neuroscience and the Oppenheimer Foundation

THURSDAY, JANUARY 29, 2009

BASIC AND TRANSLATIONAL SCIENCE SYMPOSIUM

NEUROSCIENCE RESEARCH BUILDING AUDITORIUM

8:15 am - 8:30 am INTRODUCTION AND OVERVIEW

Emeran Mayer, MD

Director, UCLA Center for Neurobiology of Stress

Roberto Peccei, PhD

Vice Chancellor of Research, UCLA

Frank Hamilton, MD, MPH

Chief, Digestive Diseases Program, NIDDK

Andrea Rapkin, MD

UCLA Department of Obstetrics and Gynecology

Co-Chair Symposium Organizing Committee

8:30 am - 8:45 am STATE OF THE CNS

Emeran Mayer, MD

Director, UCLA Center for Neurobiology of Stress

SCIENTIFIC PROGRAM

8:45 am - 10:00 am SPECIALIZED CENTER FOR NEUROVISCERAL SCIENCES & WOMEN'S HEALTH

Chairs: Lin Chang, MD and Larissa Rodriguez, MD

- 8:45 am - 9:15 am **State of the Art Lecture**
- William de Groat, PhD**
University of Pittsburgh
Nitro-Fatty Acids, Recently Discovered Signaling Molecules, Affect the Excitability of Dorsal Ganglion Neurons and Afferent Nerves in the Urinary Bladder
- 9:15 am - 10:00 am **New Investigator Presentations (10 minutes each, 15 minutes Q&A)**
- Timothy Bredy, PhD**
UCLA Department of Psychiatry and Biobehavioral Sciences
Epigenetic Mechanisms Associated with the Extinction of Conditioned Fear
- Named New Investigators:**
- Lisa Kilpatrick, PhD**
UCLA Center for Neurobiology of Stress
Impact of Mindfulness Meditation Training on the Default Network
- Jamie Maguire, PhD**
UCLA Departments of Neurology and Physiology
GABAA Receptors and Stress: Relevance to Postpartum Depression
- 10:00 am - 10:15 am **BREAK**
- 10:15 am - 12:15 pm **MINISYMPOSIUM: IDENTIFYING GENES and ENDOPHENOTYPES IN COMPLEX SYMPTOM BASED DISORDERS**
- Chairs:** Catherine Rivier, PhD and Lori Birder, PhD
- 10:15 am - 10:45 am **Jeffrey Mogil, PhD**
McGill University
What's Wrong with Animal Models of Pain?
- 10:45 am - 10:55 am Discussion
- 10:55 am - 11:25 am **Steve Horvath, PhD, DSc**
UCLA Human Genetics and Biostatistics
Integrated Weighted Gene Co-expression Network Analysis (IWGCNA) with an Application to Chronic Fatigue Syndrome
- 11:25 am - 11:35 am Discussion

- 11:35 am - 12:05 pm **Robert Bilder, PhD**
UCLA Department of Psychiatry and Biobehavioral Sciences
Neuropsychiatric Phenomics: Phenotyping Complex Syndromes on a Genome-Wide Scale
- 12:05 pm - 12:15 pm Discussion
- 12:15 pm - 2:30 pm LUNCH AND POSTER SESSION**
- 2:30 pm - 4:00 pm PROGRAM IN MIND BODY RESEARCH**
Chairs: Bruce Naliboff, PhD and David Shapiro, PhD
- 2:30 pm - 3:00 pm **State of the Art Lecture**
John Sheridan, PhD
Ohio State University
Social Stress, Glucocorticoid Resistance and Innate Immunity
- 3:00 pm - 3:10 pm **Bruce Naliboff, PhD**
Overview of CNS Mind Body Research
- 3:10 pm - 3:15 pm **Gerald Oppenheimer**
Introduction
- 3:15 pm - 4:00 pm **Data Blitz** (10 minutes each, 5 minutes Q&A)
Lisa Flook, PhD
UCLA Department of Psychiatry and Biobehavioral Sciences
Mindful Awareness Practices in Early Education Improve Executive Functions
Aditi Joshi, PhD
UCLA Center for Neurobiology of Stress
Physiological Characteristics of Traditional Chinese Medicine Based Irritable Bowel Syndrome (IBS) Sub-Groups
Ann Lowe NP, MSN
UCLA School of Nursing
Brain Mechanisms in Aromatherapy: fMRI and Mood Changes in Response to Odors in Females with Functional Abdominal Pain
- 4:00 pm - 4:15 pm Yvette Taché, PhD**
Poster Award / Wrap Up / Closing Comments

Abstracts of Presentations

SPECIALIZED CENTER FOR NEUROVISCERAL SCIENCES & WOMEN'S HEALTH

Chairs: Lin Chang, MD and Larissa Rodriguez, MD

State of the Art Lecture

Nitro-Fatty Acids, Recently Discovered Signaling Molecules, Affect the Excitability of Dorsal Ganglion Neurons and Afferent Nerves in the Urinary Bladder

William de Groat, PhD

University of Pittsburgh, Department of Pharmacology

After the discovery of the neurotransmitter function of nitric oxide ($\cdot\text{NO}$) it was recognized that this lipophilic molecule strongly influences fatty acid (FA) oxygenation at multiple levels. In addition, it was recently reported that fatty acid nitration can be induced by $\cdot\text{NO}$ derived species such as nitrogen dioxide ($\cdot\text{NO}_2$). $\cdot\text{NO}_2$ addition to the double bond of unsaturated fatty acids (oleic and linoleic acid) yields an array of regio- and stereoisomers of nitro-fatty acids (NO_2 -FAs) which display both cGMP-independent and receptor dependent signaling actions as well as robust electrophilic reactivity. NO_2 -FAs are formed by oxidative inflammatory reactions and have been shown to have anti-inflammatory effects in sub-micromolar concentrations. Multiple mechanisms have been implicated in the anti-inflammatory effects including inhibition of the expression of iNOS and cytokine secretion as well as inhibition of NF- κ B activation.

We have examined the acute effects of nitro-octadecenoic acid (nitro-oleic acid, OANO_2), a potent NO_2 -FA, on the electrical properties of rat dorsal root ganglion (DRG) neurons in culture and on afferent nerves in bladder smooth muscle strips. Whole cell patch clamp recording revealed that OANO_2 reduces the membrane potential and induces inward currents in 65% of lumbosacral DRG neurons. The effect of OANO_2 on membrane potential and membrane currents was concentration-dependent, occurring at >5 nM and reaching a maximum at 50-500 nM. In OANO_2 -responsive neurons, 45% also responded to capsaicin (CAPS), a TRPV1 agonist and 60% responded to allyl isothiocyanate (AITC), a TRPA1 agonist, with inward currents. 50% of these neurons were sensitive to both CAPS and AITC. OANO_2 -induced currents and TRPV1 and TRPA1 agonist-induced currents were additive. These additive currents were reversed by TRPV1 or TRPA1 antagonists and dithiothreitol, a reducing agent that interferes with OANO_2 -protein thiol reactions. These data indicate that OANO_2 activates TRPV1 and TRPA1 channels in DRG neurons. Higher concentrations of OANO_2 (>500 nM) also reduced action potential (AP) overshoot, increased AP threshold and duration and inhibited firing induced by depolarizing current pulses.

In bladder strips from neonatal rats OANO_2 enhanced spontaneous contractile activity and increased baseline pressure. The effects were blocked by a TRPA1 receptor antagonist and also by a combination of neurokinin 1, 2 and 3 receptor antagonists. A TRPV1 receptor antagonist which blocked the excitatory effect of capsaicin did not alter the response to OANO_2 . It is concluded that OANO_2 which is a strong electrophile can activate TRPA1 receptors in bladder afferent nerves and release tachykinins which in turn stimulate the bladder smooth muscle.

In summary, OANO₂, an extracellular signaling molecule released at sites of inflammation has prominent modulatory actions on afferent neurons. These actions may contribute to the anti-inflammatory and anti-nociceptive effects of NO₂-FAs.

New Investigator Presentations

Epigenetic Mechanisms Associated with the Extinction of Conditioned Fear

Timothy Bredy, PhD

UCLA Department of Psychiatry and Biobehavioral Sciences

Background: Epigenetics refers to the molecular processes that determine how cellular traits are established and inherited without a change in DNA sequence. These ancient mechanisms of cellular memory also orchestrate gene expression in the adult brain, and recent findings suggest a role in the etiology of psychiatric disease. The apparatus that controls access to DNA by way of chromatin therefore represents an important interface between environmental signals, activation and repression of genomic responses, and adaptive behavior. As of yet, however, the precise epigenetic mechanisms by which fear memories persist and, perhaps more importantly, can be inhibited by extinction are poorly understood. Previously, we demonstrated that histone modifications are associated with the extinction of conditioned fear and pharmacotherapy directed towards epigenetic modifying enzymes can facilitate fear extinction (Bredy et al, 2007; 2008).

Methods and Results: Using a pre-clinical model of fear-related anxiety disorder and its treatment, we have continued to elucidate the epigenetic signature that is unique to fear extinction. Protein antibody microarray was performed on tissue derived from fear conditioned versus extinction-trained mice, and distinct global epigenetic regulatory proteins that can be differentiated both by learning protocol and by neuroanatomical location were revealed. For example, the histone acetyltransferase p300/CBP-associated factor (PCAF) is increased two hours after extinction learning in both hippocampus and prefrontal cortex. Conversely, methyl CpG binding protein 2 (MeCP2) is increased in the prefrontal cortex, but decreased in hippocampus, after extinction learning. Using a genome-wide approach (MeDIP-CHIP), we have also identified distinct sets of gene promoters in prefrontal cortex that show variations in DNA methylation/demethylation after extinction relative to fear conditioning (e.g. D4 receptor).

Discussion: The challenge is to determine the functional significance of a dynamic epigenome in the maintenance of extinction memory. By elucidating the epigenome as a fundamental regulatory mechanism involved in the stabilization and persistence of long-term fear memories and their suppression through extinction, we're making important progress toward understanding the role of inhibitory learning in the treatment of anxiety disorders.

Impact of Mindfulness Meditation Training on the Default Network

Lisa Kilpatrick, PhD

UCLA Center for Neurobiology of Stress

Introduction: The practice of meditation has beneficial effect on physical and psychological health, as well as positive regulatory effects on physiological processes. Presumably these effects result from changes in underlying brain processes. Mindfulness-Based Stress Reduction (MBSR) training is of particular research interest because it utilizes a traditional practice applied in a well-documented, standardized contemporary format. As part of a larger study of mindfulness meditation and pain processing, we investigated how mindfulness meditation

impacts default mode network (DMN) activity. The DMN is a set of brain regions that are metabolically active in the absence of goal-directed activity, and are disrupted in a number of mental disorders. We hypothesized that the DMN of MBSR-trained subjects, relative to wait list controls, would demonstrate greater coherence with regions previously associated with a present moment, experiential self-focus and less coherence with regions associated with a narrative self-focus.

Methods: 12 healthy, meditation naive female subjects were scanned following an MBSR course and compared to 8 wait list controls. Subjects were asked to close their eyes and be mindfully aware of their surroundings during a 5 minute baseline functional magnetic resonance imaging (fMRI; 1.5 T) scan. Group independent component analysis was performed to investigate training-related changes among DMN brain regions during mindful rest.

Results: MBSR-trained subjects and controls had significant differences in the default mode network. MBSR-trained subjects, compared to controls, showed significantly increased DMN connectivity with the left dorsal and inferior lateral prefrontal cortex (BA10), left angular gyrus (BA39), left inferior parietal lobule (BA40), and bilateral precuneus (BA7) and decreased DMN connectivity with bilateral hippocampus, right dorsolateral prefrontal cortex (DLPFC; BA46) and bilateral posterior cingulate cortex (BA23).

Conclusions: MBSR students learn to generate a state of focused awareness on the present moment with curiosity and equanimity. DMN differences between MBSR-trained subjects and controls suggest a training-induced shift from a greater coherence with regions supporting ruminative, narrative-based processing (hippocampus, BA23) to a greater coherence with regions supporting present moment, experiential processing (BA10, BA39, BA40). Greater coherence with DLPFC in controls may reflect greater effort to resist narrative mind wandering. In summary, MBSR-training appears to induce changes in the nature of self-referential thought processes occurring in the absence of goal-directed behavior, facilitating present-centered self-awareness and reducing ruminative type thinking.

GABAA Receptors and Stress: Relevance to Postpartum Depression

Jamie Maguire, PhD

UCLA Departments of Neurology and Physiology

The postpartum period is remarkably vulnerable to the manifestation of mood disorders likely due to the changes in neurosteroid levels which alters neuronal excitability. Robust changes in neurosteroid levels throughout pregnancy and postpartum profoundly alter GABA_A receptor (GABA_AR) structure and function. Downregulation of GABA_ARs during pregnancy may be necessary to prevent sedation and/or anaesthesia during pregnancy and timely recovery of inhibition may be required to prevent abnormal behaviors in the postpartum period. Mice deficient in GABA_AR δ subunits (*Gabrd*^{+/-} and *Gabrd*^{-/-}), in which the postpartum restoration of inhibition is likely to be impaired, exhibit depression, anxiety, and abnormal maternal behavior postpartum resulting in reduced pup survival. Impaired GABAergic regulation of the HPA axis in *Gabrd*^{+/-} and *Gabrd*^{-/-} mice may underlie the anxiety manifested during the postpartum period. Consistent with this hypothesis, the stress response is exacerbated in *Gabrd*^{-/-} mice, resulting in increased corticosterone levels. In addition, wild type mice subjected to ultramild stress throughout pregnancy or treated with exogenous corticosterone exhibit depression, abnormal maternal behaviors and decreased pup survival. These data suggest that dysregulation of the HPA axis may play a role in postpartum depression.

MINISYMPOSIUM: IDENTIFYING GENES and ENDOPHENOTYPES IN COMPLEX SYMPTOM BASED DISORDERS

Chairs: Harry Catherine Rivier, PhD and Steve Horvath, PhD, ScD

What's Wrong with Animal Models of Pain?

Jeffrey Mogil, PhD

McGill University

Researchers studying pain in animal models face a conundrum. Any number of reliable assays are available, but the mechanisms underlying pain processing in these assays are more and more thought to be of uncertain relation to mechanisms underlying clinical pain. However, the production of more clinically relevant pain states in laboratory animals yields few usable and selective dependent measures. As a result, most basic science pain researchers continue to rely on the measurement of reflexive, evoked hypersensitivity responses after neuropathic or inflammatory injury, whereas clinical pain in humans features much spontaneous pain and an important cognitive and emotional overlay. In addition to the disconnect between clinical symptoms and animal measures, there is a disconnect between the clinical epidemiology of pain and the types of pain being studied by basic scientists. Much attention is focused on neuropathic pain in the laboratory, but very little attention on much more common pain disorders like headache, arthritis and back pain. We have recently attempted to develop an "ethological" approach to animal models of common pain pathologies, involving systematic and rigorous analysis of videotaped spontaneous mouse behaviors. I will describe some recent successes, both prominently featuring sex differences: new mouse models of migraine and vestibulodynia.

Integrated Weighted Gene Co-expression Network Analysis (IWGCNA) with an Application to Chronic Fatigue Syndrome

Steve Horvath, PhD, DSc

UCLA Human Genetics and Biostatistics

Weighted Gene Co-expression Network Analysis (WGCNA) can effectively integrate gene expression and trait data to identify pathways and candidate biomarkers. Here we show that the additional inclusion of genetic marker data allows one to characterize network relationships as causal or reactive in a chronic fatigue syndrome (CFS) data set.

We combine WGCNA with genetic marker data to identify a disease-related pathway and its causal drivers, an analysis which we refer to as "Integrated WGCNA" or IWGCNA. Specifically, we present the following IWGCNA approach: 1) construct a co-expression network, 2) identify trait-related modules within the network, 3) use a trait-related genetic marker to prioritize genes within the module, 4) apply an integrated gene screening strategy to identify candidate genes and 5) carry out causality testing to verify and/or prioritize results. By applying this strategy to a CFS data set consisting of microarray, SNP and clinical trait data, we identify a module of 299 highly correlated genes that is associated with CFS severity. Our integrated gene screening strategy results in 20 candidate genes. We show that our approach yields biologically interesting genes that function in the same pathway and are causal drivers for their parent module. We use a separate data set to replicate findings and use Ingenuity Pathways Analysis software to functionally annotate the candidate gene pathways. We show how WGCNA can be combined with genetic marker data to identify disease-related pathways and the causal drivers within

them. The systems genetics approach described here can easily be used to generate testable genetic hypotheses in other complex disease studies.

References:

1) Presson AP, Sobel EM, Papp JC, Suarez CJ, Whistler T, Rajeevan MS, Vernon SD, Horvath S (2008) Integrated weighted gene co-expression network analysis with an application to chronic fatigue syndrome. *BMC Systems Biology* 2008, 2:95; 2) Horvath S, Dong J (2008) Geometric Interpretation of Gene Coexpression Network Analysis. *PLoS Comput Biol* 4(8): e1000117; 3) Zhang B, Horvath S (2005) "A General Framework for Weighted Gene Co-Expression Network Analysis", *Statistical Applications in Genetics and Molecular Biology*: Vol. 4: No. 1, Article 17.

Neuropsychiatric Phenomics: Phenotyping Complex Syndromes on a Genome-Wide Scale

Robert Bilder, PhD

UCLA Department of Psychiatry and Bibehavioral Sciences

This lecture will describe the new trans-discipline of “phenomics” – the systematic study of phenotypes on a genome-wide scale – and focus on how this emerging strategy, if successful, may transform research on a variety of complex clinical disorders. The *Consortium for Neuropsychiatric Phenomics* at UCLA, one of nine Interdisciplinary Research Consortia across all biomedicine supported by the NIH Roadmap Initiative, serves as an example of the phenomics strategy actively being applied to advance understanding of the causes and treatments of neuropsychiatric syndromes. The lecture will describe the revolutionary impact of genome-wide association studies on gene discovery for complex diseases, and focus on challenges encountered in neuropsychiatry research but that likely apply to other clinical entities as well. In contrast to prior work that has emphasized diagnosis as a target phenotype, phenomics instead focuses on discerning complex phenotypes expressed across multiple levels of observation (from protein, through cellular systems and signaling pathways, to neural systems, cognitive profiles, and discrete symptom clusters) that may not honor conventional diagnostic boundaries. The most fruitful phenotypes for research may indeed cross species boundaries. New informatics and analytic strategies are being used to represent, visualize, and test multi-level hypotheses that could help define novel dimensions of vulnerability to disease, and offer previously unanticipated directions for treatment development. The implications of this work for future diagnosis and treatment of complex disorders will be discussed.

PROGRAM IN MIND BODY RESEARCH

Chairs: Bruce Naliboff, PhD and David Shapiro, PhD

State of the Art Lecture

Social Stress, Glucocorticoid Resistance and Innate Immunity

John Sheridan, PhD

Ohio State University

Repeated exposure to threatening stimuli can result in prolonged anxiety. Although many of the neurobiological processes responsible for anxiety have been defined, the role of stress-induced proinflammatory cytokines in the induction of prolonged anxiety-like behavior has not been systematically studied. The overarching hypothesis of this research is that social stress-induced increase in proinflammatory cytokines leads to prolonged anxiety-like behavior. A model of repeated social defeat (RSD) has been used to elucidate the pathways and mechanisms by which social stress affects central and peripheral physiology and leads to prolonged anxiety-like behavior. Neuroimmune products such as catecholamines and cytokines are well known to influence various physiological outcomes including immune function and behavioral responses. Additionally, specific activation of distinct brain regions can be noted in animals displaying fear or anxiety-like behavior. The model of stress employed in these studies causes significant increases in tissue norepinephrine and proinflammatory cytokine production. Hyperinflammation in RSD mice can be attributed, in part, to RSD-induced glucocorticoid resistance in distinct monocyte populations (CD11b+ and CD11c+ cells). Further, RSD-affected mice display prolonged anxiety-like behavior. This research tests the hypotheses that; 1) The development of prolonged anxiety-like behavior, in response to repeated social defeat, is associated with central and peripheral catecholamine responses and enhanced proinflammatory cytokine gene expression. 2) Catecholamines released in peripheral lymphoid tissues result in the development of glucocorticoid (GC) insensitive cells and a subsequent enhancement of proinflammatory cytokine responses that activate the CNS and alter behavior.

Mindful Awareness Practices in Early Education Improve Executive Functions

Lisa Flook, PhD

UCLA Department of Psychiatry and Biobehavioral Sciences

In two pilot studies we evaluated a program of mindful awareness practices (MAPs) as a tool to promote attention and emotion regulation in pre-school and elementary school age children. MAPs activities are a preventative mind-body approach to enhance awareness through exercises that promote an observant stance towards one's feelings, thoughts, and body states in the present moment. The pilot studies showed improvement in executive functions among participants compared to controls following an 8-week MAPs training program at school. We are currently conducting a replication and extension of the preschool study using objective measures of attention and biological stress regulation.

Physiological Characteristics of Traditional Chinese Medicine Based Irritable Bowel Syndrome (IBS) Sub-Groups

Aditi Joshi, PhD

UCLA Center for Neurobiology of Stress

Background: It is well known that patients with IBS also have a high rate of co-morbid extra-intestinal symptoms. TCM views all illnesses as a pattern of dysregulation and therefore would predict that subgroups of IBS patients may have differing characteristics, including the pattern of extra-intestinal symptoms, pain sensitivity, and stress responses and that these may reflect different IBS pathophysiology.

Aim: To determine reliability of TCM diagnoses across practitioners and stress reactivity and pain sensitivity of Traditional Chinese Medicine (TCM) based IBS sub-groups.

Methods: To date 30 female IBS patients were evaluated by two TCM practitioners for their primary pattern of dysregulation; “*Liver Qi Stagnation*” (EXCESS pattern), “*Spleen Qi Deficiency*” (DEFICIENCY pattern), or a combination of both patterns (OVERLAP). They also filled out a TCM symptom questionnaire (TCMIQ). During a laboratory session autonomic and psychological measures of stress were recorded during physical and psychological stress tasks and sensitivity to cutaneous heat and pressure pain was measured. We hypothesized ‘EXCESS’ individuals would show more autonomic reactivity characterized by increase in blood pressure, heart-rate and skin conductance during stress as compared to those with a ‘DEFICIENT’ pattern as well as showing an increase in pain sensitivity.

Results: In this preliminary data set there was excellent agreement on the diagnosis by the two doctors (29/30). No significant differences were found in autonomic reactivity across two groups based on the diagnosis of TCM doctors however no pure DEFICIENCY patients have been studied. Patients with greater numbers of deficiency symptoms compared to those with few deficiency symptoms based on the TCMIQ have higher diastolic blood pressure ($p=.02$) and higher systolic blood pressure ($p=.09$) during the stress task but not rest. Those with higher deficiency symptoms also show an increased pain sensitivity to higher pressures.

Conclusion: This preliminary analysis shows an increase in deficiency symptoms is associated with greater autonomic stress responses and pain sensitivity in IBS. Further examination of the other study measures and recruitment of patients with a pure DEFICIENCY pattern will be help determine how the two TCM patterns may differ on these measures.

Brain Mechanisms in Aromatherapy: fMRI and Mood Changes in Response to Odors in Females with Functional Abdominal Pain

Ann Lowe NP, MSN

UCLA School of Nursing

Aromatherapy is an ancient practice used to improve moods and enhance spiritual experiences. More recently odor has been shown to impact the experience of pain. Irritable bowel syndrome (IBS) is a stress-mediated functional pain syndrome with a high incidence of comorbid psychological and chronic pain pathologies. The purpose of this study is to investigate the role of olfaction in modulating mood and cortical response to emotional stimuli in female subjects with irritable bowel syndrome (IBS), a stress-sensitive functional bowel disorder with altered perception of visceral sensations, and an enhanced response to emotionally evocative stimuli. Previous studies have demonstrated that odors can impact pain by modulating mood, but have not yet investigated the role of smell in IBS symptom perpetuation. As the olfactory bulb is highly interconnected with the amygdala, a region of fear conditioning, and smell is central to taste, odor may play a role in symptom regulation. In recent research, IBS subjects, when exposed to an emotional faces protocol, demonstrated increased activation of paralimbic

structures and diminished recruitment of corticolimbic inhibitory mechanisms to downregulate emotional generating circuits (Mayer & colleagues, 2007). This study utilizes a 2 group design (N=18; IBS women and normal controls) and an analogous protocol using fMRI, but will add a pleasant odor, unpleasant odor, and clean air. As data collection was completed on January 23, 2009, this talk will address preliminary findings, including a case presentation of an IBS and a control subject.

Biosketches of Speakers

Robert Bilder, PhD

UCLA Department of Psychiatry and Biobehavioral Sciences

Dr. Bilder is currently Chief of Medical Psychology – Neuropsychology at the Jane & Terry Semel Institute for Neuroscience and Human Behavior, Michael E. Tennenbaum Family Professor of Psychiatry & Biobehavioral Sciences in the David Geffen School of Medicine, and Professor of Psychology in the College of Letters and Science at UCLA.

Dr. Bilder received a bachelor's degree in Biology and Psychology from Columbia College of Columbia University, and a doctorate in Psychology from City College of the City University of New York, where he specialized in human neuropsychology. He did his Internship in Neuropsychology at the New York State Neurological Institute of Columbia-Presbyterian Medical Center. Before joining UCLA in 2002, Dr. Bilder held faculty appointments at Columbia College of Physicians & Surgeons and the Albert Einstein College of Medicine. He was Chief of Neuropsychology at North Shore – Long Island Jewish Medical Center, and Associate Director of the Center for Advanced Brain Imaging at the Nathan Kline Institute. Dr. Bilder is a diplomate in Clinical Neuropsychology, and currently serves on the Boards of Directors for the American Academy of Clinical Neuropsychology, and the American Association of Postdoctoral Programs in Clinical Neuropsychology.

Dr. Bilder is has been engaged for over 25 years in research on the brain bases of mental illness. Dr. Bilder's current research focuses on transdisciplinary and translational research, and particularly on developing the new discipline of "phenomics" to enable the systematic study of neural system phenotypes on a genome-wide scale (see www.phenomics.ucla.edu). He directs the Consortium for Neuropsychiatric Phenomics, supported by the NIH Roadmap Initiative as part of its theme "research teams of the future", to develop and deploy innovative methods for studying neural system phenotypes that will offer more promising targets for gene and drug discovery. Dr. Bilder also directs the Tennenbaum Center for the Biology of Creativity, and is Co-Director of an NIMH-sponsored Center for Intervention Development and Applied Research (CIDAR) at UCLA, focusing on Translational Research to Enhance Cognitive Control particularly in children and adolescents.

Timothy Bredy, PhD

UCLA Department of Psychiatry and Biobehavioral Sciences

Timothy Bredy is a postdoctoral fellow in the UCLA Semel Institute for Neurosciences and Behavior as well as in the Department of Medical Genetics, at the University of British Columbia where he currently holds Canadian Institute of Health Research and Michael Smith Foundation for Health Research (MSFHR) postdoctoral fellowships. He earned his undergraduate degrees (BA and BSc) at the Dalhousie University in Halifax and his doctorate in the Department of Neurology and Neurosurgery at McGill University in Montreal. Dr. Bredy is using state-of-the-art technology to investigate the epigenetic machinery involved in regulating critical gene targets during the acquisition and extinction of conditioned fear. Dr. Bredy hopes his findings will provide insight into the molecular basis of long-term storage of fear-related memories, relapse

and its prevention, and that this research will ultimately contribute to the design of novel pharmacotherapeutic approaches for fear-related anxiety disorders.

William de Groat, PhD
University of Pittsburgh

Dr. de Groat is Professor of Pharmacology at the University of Pittsburgh. He earned his bachelor's (pharmacy) and master's (pharmacology) degrees at the Philadelphia College of Pharmacy and Science, and his doctorate (pharmacology) at the University of Pennsylvania Medical School. He subsequently received postdoctoral training at the University of Pennsylvania and at the Australian National University before joining the faculty of the University of Pittsburgh Medical School in 1968. Dr. de Groat is a Fellow of the American Association for the Advancement of Science and past-President of the 4th World Health Organization International Consultation on Incontinence. He is the recipient of many honors and awards both for his teaching and for his research. The list includes the "Golden Apple" Award of the American Medical Students Association in 1974, 1978, 1980, 1983 and 1988; the Lifetime Achievement Award from the Urodynamics Society in 1995; the Pharmacia-ASPET Award for Experimental Therapeutics from the American Society for Pharmacology and Experimental Therapeutics in 2003; and the Reeve-Irvine Research Medal for research in the field of spinal cord injury awarded by the University of California in 2007. Dr. de Groat's international renown is reflected further in a long list of invited lectureships including the DeLourme Distinguished Lecturer, Biennial ICSCI Conference, Paris, in 1991; the Sir Ludwig Guttman Lecturer, International Congress of Paraplegia, Ghent, in 1993; the Grass Traveling Scientist Lecturer, sponsored by Society for Neuroscience in 1994; the Elsevier-JANS Lecturer at the Millennium Congress of the International Society for Autonomic Neuroscience, London, in 2000; and most recently the British Urological Foundation Keynote Lecturer at the Annual Meeting of the British Association of Urological Surgeons in 2008.

Dr. de Groat is interested in the autonomic nervous system and the neural regulation of pelvic visceral functions. His current studies focus on the reflex control of the urogenital tract and the mechanisms underlying transmission at central and peripheral autonomic synapses. These experiments are designed to examine (1) the neurotransmitters in the reflex pathways, (2) neuroplasticity during postnatal development or following neural injury, (3) the neural pathways responsible for the detection of visceral pain, and (4) the actions of drugs used to treat urogenital dysfunction. Experiments are conducted on a variety of preparations ranging from intact animals to isolated tissues, like spinal cord slices and dissociated neurons.

Lisa Flook, PhD
UCLA Department of Psychiatry and Biobehavioral Sciences

Lisa Flook received her PhD in clinical psychology from UCLA. She is currently a research scientist with the Mindful Awareness Research Center at UCLA. Her research has focused on how family and school experiences shape children and adolescents' adjustment. She is interested in prevention and early intervention strategies to promote well-being in daily life. Dr. Flook is involved in research on the applications of mindfulness-based practices for clinical and non-clinical populations. She is currently investigating the utility and feasibility of introducing mindfulness practices to children in educational settings.

Steve Horvath, PhD, DSc

UCLA Human Genetics and Biostatistics

Steve Horvath is an Associate Professor in Human Genetics and Biostatistics at the University of California, Los Angeles. He has a PhD in mathematics and a DSc in Biostatistics. Dr Horvath's research area lies at the intersection of biostatistics, computational biology, bioinformatics, mathematics, statistical genetics, machine learning, cancer genetics, systems biology, genetic epidemiology, and complex disease mapping. He has published extensively on a) data mining methods for health research, b) networks methods and systems biology, and c) genetic marker association tests. Recently, Dr Horvath's group has developed and applied weighted gene coexpression network analysis (WGCNA), which is a systems biologic data analysis method. Statistically speaking, WGCNA can be used as data reduction technique (related to oblique factor analysis), as gene clustering method (fuzzy clustering), as gene screening method (pathway based gene screening), as framework for integrating genomic data (based on weighted correlations between quantitative variables), and as data exploratory technique.

Aditi Joshi, PhD

UCLA Center for Neurobiology of Stress

Aditi Joshi earned her Bachelors of Ayurvedic Medicine and Surgery and her Masters in Ayurvedic Medicine from the University of Pune, India. She completed her PhD from the University of Oregon. Currently, she is a post-doctoral researcher at Neuropsychiatry Institute, UCLA. Her research interests are Alternative and complementary medicine and stress.

Lisa Kilpatrick, PhD

UCLA Center for Neurobiology of Stress

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 Neurovisceral Sciences and Women's Health. Her research focuses on the altered central and autonomic nervous system processes in functional pain disorders such as Irritable Bowel Syndrome (IBS) as well as the potential therapeutic effects of pharmacological and alternative treatments. Current projects include prepulse modulation of the acoustic startle response in IBS, interstitial cystitis, and fibromyalgia patients; the effect of slow-paced breathing on limbic reactivity in IBS patients; and the effect of mindfulness meditation on neural network activity during resting conditions. She is dedicated to exploring sex differences in nervous system processes as an important step towards tailoring therapies to individual neurobiologies.

Ann Lowe NP, MSN

UCLA School of Nursing

Ann Lowe is a clinician and a researcher with over 20 years experience as a nurse practitioner. Throughout her career, she has performed as a clinical specialist, a manager of a geriatric center, a principal member of a geriatric assessment team, and a nurse practitioner for a cardiologist and rheumatologist. Being primarily focused on outpatient care, her emphasis has been the management of chronic health problems. She is a graduate of the California College of Ayurveda and, as an Ayurvedic clinical specialist, opened up an integrative practice called,

“East Meets West Health Care”. Ann is currently a doctoral candidate at the UCLA School of Nursing, and a recipient of an Oppenheimer Seed grant for Complementary and Alternative Medicine, the Sigma Theta Tau nursing honor society research grant, and an ALBMC award. Ann is committed to integrative medicine, in which the ideas of ancient health care systems are understood using the methods of modern science.

Jamie Maguire, PhD

Department of Neurology, UCLA

Dr. Jamie Maguire is an Assistant Researcher within the Department of Neurology at The University of California, Los Angeles (UCLA) David Geffen School of Medicine. Dr. Maguire's research focuses on investigating the role of steroid hormones and their neuroactive derivatives, or neurosteroids, on neuronal excitability. Her research on the impact of steroid hormones on GABAA receptors has altered the way scientists think about steroid hormone-linked neurological disorders, such as premenstrual syndrome (PMS), premenstrual dysphoric disorder (PMDD), catamenial epilepsy, and postpartum depression. Dr. Maguire's research shows that fluctuations in neurosteroid levels, such as over the ovarian cycle, following stress, and during pregnancy, alter the structure and function of GABAA receptors leading to changes in neuronal excitability. Her research has received widespread acclaim from mainstream as well as academic audiences. Dr. Maguire has received several competitive grants to further research in the area of hormone regulation of GABAergic inhibition from the American Epilepsy Foundation of America, the National Institutes of Health, and a Pilot and Feasibility Award from the Center for Neurobiology of Stress at UCLA. She has also been the recipient of many awards for her ongoing research, including Investigator's Workshop poster award winner at the American Epilepsy Society meetings in 2005 and 2006, the Named New Investigator at the Center for Neurovisceral Sciences and Women's Health, and recipient of a Young Investigator's Award from the American Epilepsy Society. She has also been an invited speaker at many provocative meetings, such as the Cold Spring Harbor Laboratory meeting on “GABAergic Inhibition” and the “Inhibition in the CNS” Gordon Research Conference.

Jeffrey Mogil, PhD

McGill University

Jeffrey S. Mogil was born in Toronto, Ontario, Canada in 1966. He received a B.Sc. (Honours) in Psychology from the University of Toronto in 1988, and a Ph.D. in Neuroscience from UCLA in 1993. After a postdoctoral fellowship in Portland, OR from 1993 to 1996, he joined the faculty of the Dept. of Psychology at the University of Illinois at Urbana-Champaign. He moved to McGill University in 2001, and is currently the E.P. Taylor Professor of Pain Studies (a Chair previously occupied by Dr. Ronald Melzack) and the Canada Research Chair in the Genetics of Pain (Tier I).

Dr. Mogil has made seminal contributions to the field of pain genetics and is the author of most major reviews of the subject, including an edited book, *The Genetics of Pain* (IASP Press, 2004). He is also a recognized authority in the fields of sex differences in pain and analgesia, and algesiometric testing in the laboratory mouse. Dr. Mogil is the author of over 130 articles and book chapters since 1992, and has given over 150 invited lectures in that same period. He holds or has held funding from the U.S. National Institutes of Health, the Canadian Institutes for Health Research, the Canada Foundation for Innovation, Genome Canada, Neuroscience Canada and the pharmaceutical/biotech industry. He is the recipient of numerous awards,

including the Neal E. Miller New Investigator Award from the Academy of Behavioral Medicine Research (1998), the John C. Liebeskind Early Career Scholar Award from the American Pain Society (1998), the Patrick D. Wall Young Investigator Award from the International Association for the Study of Pain (2002) and the Early Career Award from the Canadian Pain Society (2004).

John Sheridan, PhD
Ohio State University

Dr. Sheridan is Professor of both Oral Biology and Molecular Virology, Immunology & Medical Genetics at Ohio State University, where he also serves as Associate Dean for Research in the College of Dentistry. Dr. Sheridan received a bachelor's degree in Biological Sciences from Fordham University, and master's and doctorate degrees in microbiology from Rutgers University. Before joining the faculty at Ohio State in 1984, he was on the faculty of Johns Hopkins. Dr. Sheridan is a Fellow, American Association for the Advancement of Science. He also serves on the editorial board of Brain, Behavior, and Immunity.

Dr. Sheridan's laboratory uses cellular and molecular techniques to study viral pathogenesis, anti-viral immunity and wound healing. An integrative physiological approach is used to study interactions among the nervous, endocrine and immune systems. Animal models of experiment viral infections (including influenza, herpes simplex and coxsackie viruses) have been adapted to study the effect of neuroendocrine responses on cytokine and chemokine gene expression. In addition, the effects of age and stress on gene expression during infection and wound healing are currently being studied in humans and in animal models. His current research interests include the following

- Viral immunology, regulation of T cell responses during viral infection, cytokine and chemokine gene expression during infection (influenza, herpes simplex and coxsackie viruses).
- Neuroendocrine-immune interactions; stress-induced modulation of anti-viral immune responses and viral pathogenesis.
- Aging and the immune response to viral infection.
- Aging and gene expression during wound healing. Patterns and kinetics of gene expression for cytokines, chemokines and growth factors during cutaneous wound healing.

Abstracts of Posters

Basic and Translational

1.

Spinal Microglia Activation Is Involved in the Development of Chronic Stress Induced Visceral Hyperalgesia and Associated Spinal NK1 Receptor Up-Regulation in the Rat

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Background: Increasing evidence supports an important role of spinal microglia (involving MAPK and NFκB activation) in animal models of chronic somatic pain. Chronic water avoidance stress (WA) in rats leads to sustained visceral hyperalgesia, and this is associated with increased expression of NK1 receptors (NK1R) on dorsal spinal neurons (Gastroenterology 2006, 130(6):1729-42). The aim of this study was to characterize the role of activated spinal microglia in stress-induced visceral hyperalgesia and associated NK1R over expression in the spinal cord.

Methods: Male Wistar rats were equipped with electrodes for the recording of abdominal electromyographic (EMG) activity in response to colorectal distension (CRD) and/or with chronic intrathecal catheters connected to osmotic mini-pumps for chronic drug delivery. Some rats received an acute intrathecal (IT) injection of the microglia activating factor fractalkine (40 ng) or vehicle, preceded 1 hour before, by injection of the microglia inhibitor minocycline (100 mg, IT) or vehicle. Other groups of rats (n=16) were exposed to WA or sham WA, 1 hour daily for 10 consecutive days and received continuous IT infusion with minocycline (120 mg/day), the MAPK p38 inhibitor SB203580 (4mg/day) or vehicle. Groups of 8 were tested for the EMG response to CRD at baseline and post WA stress at day 11. The other groups of 8 were sacrificed at day 11 and L6S1 spinal cord samples were collected and processed for western blotting for the phosphorylated MAPK p38 (P-p38, previously shown to be increased in activated microglia), NK1R and IκBα (decreased when NFκB is activated).

Results: Fractalkine increased EMG response to CRD (P<0.05 compared with vehicle), and this response was significantly reduced by minocycline (P<0.05). WA induced increased EMG response to CRD compared with sham WA. This effect was blocked by minocycline or SB203580 (P<0.05). WA rats showed increased levels of proteins for P-p38, NK1R and reduced IκBα in spinal extracts (P<0.05) compared with sham WA. Minocycline significantly (P<0.05) blocked WA-induced changes of P-p38, NK1R, and IκBα while treatment with SB203580 blocked WA-induced increase of P-p38 and decreased IκBα (P<0.05) but did not affect WA-induced NK1R increased expression.

Conclusion: This is the first demonstration that activation of spinal microglia, triggered by a microglia agonist or by chronic WA stress, plays a role in the development of visceral hyperalgesia and contributes to stress-induced NK1R up regulation. The role of p38 or other MAPKs in the modulation of NK1R expression needs to be further investigated.

2. Pilot Study on the Spontaneous Plasticity of Lower Urinary Tract Functions After Unilateral Lumbosacral Ventral Roots Avulsion

H-Y Chang, L Havton

Department of Neurology, UCLA

Injuries to peripheral nerves and lumbosacral nerve roots may cause impairments of bladder, bowel and reproductive functions. For instance, women undergoing regional anesthesia in connection with obstetric procedures may be at risk result of cauda equina injuries and childbirth may result in crush lesions of the pudendal nerve. Both cauda equina injuries and pudendal nerve lesions result in a lower motoneuron symptom complex, which includes partial or complete denervation of the lower urinary tract and results in bladder areflexia and urinary retention. Unfortunately, both cauda equina injury and pudendal nerve injuries are associated with poor recovery of lower urinary tract (LUT) function. We have developed a clinically relevant model of CE/CM SCI in rats that involves the avulsion, or tearing, of the lumbosacral (L6-S1) ventral motor roots from one side of the spinal cord (i.e., ventral root avulsion, or VRA). These ventral roots provide motor and autonomic function to the pelvic muscles, bladder, and bowels. In this pilot study, we examined the cystometry and external urethral sphincter (EUS) electromyography (EMG) in the animals at 8 weeks after unilateral L6-S1 VRA injury. Our preliminary data suggested that the LUT functions were affected in the rats with unilateral L6-S1 VRA injury. In VRA injured rats (n=3), the inter-contraction interval and contraction duration of a single voiding cycle as well as the amplitude of EUS EMG activity increased compared to control rats (n=3). Voiding efficiency in VRA injured rats (47%) decreased compared to control rats (80%). These preliminary results showed strong possibilities of plasticity on the LUT functions in the rats at 8 weeks after unilateral VRA injury. Further experiments need to be examined to reveal the possible mechanisms for synapse plasticity as well as the recovery of LUT functions after lower motoneuron lesions either acutely or chronically in a rat model.

3. Impairment of Hippocampus-Dependent Learning and Memory During Diestrus in Mice

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The delta-subunit of the GABA_AR is highly expressed in the dentate gyrus of the hippocampus. It is predominantly located extrasynaptically in the dendrites of granule cells where it is thought to mediate a tonic inhibitory current that is highly sensitive to spillover of GABA from nearby synapses. The delta-subunit is also highly sensitive to neurosteroids such as the progesterone metabolite allopregnanalone. In female mice, the expression level of the delta-subunit within the dentate gyrus fluctuates across the estrus cycle. Its expression is highest during diestrus resulting in enhanced tonic inhibition specifically in the dentate gyrus and decreased kainic acid induced seizure severity. Conversely, during estrus its expression is low resulting in decreased tonic inhibition and enhanced seizure severity. Previous work in our lab has shown that female delta-GABA_AR KO mice show an enhancement in hippocampus-dependent trace but not hippocampus-independent delay fear conditioning whereas male delta-GABA_AR KO's and wildtypes do not differ on either task. Based on the analysis of individual freezing scores in that

study the enhanced trace conditioning in female delta-GABA_AR KO mice seems to be due to the absence of mice that failed to learn. In other words, wild type females showed a range of freezing levels with some mice exhibiting very low levels of freezing suggesting that these particular mice failed to learn. None of the female delta GABA_AR KO mice exhibited low freezing levels suggesting they all successfully learned the tone-shock association. This previous study did not control for phase of the estrus cycle. The current study, therefore, tested the hypothesis that the “failures to learn” in female wild-type mice were from mice that were trained during diestrus when delta-subunit expression and progesterone mediated inhibition is high. Results indicate impaired acquisition and retention of context and trace fear conditioning during diestrus, suggesting that enhanced tonic inhibition in the dentate gyrus during diestrus impairs hippocampus-dependent fear learning and memory.

4. Nesfatin-1 Immunoreactive Brain Nuclei Are Activated by Restraint and Abdominal Surgery in Rats

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Nesfatin-1, the newly discovered peptide that was shown to reduce food intake after central injection, is widely distributed in the rat brain. This implies other, yet unknown functions besides regulation of food intake. Recent studies described increased anxiogenic behavior induced by central nesfatin-1 injection and delay of gastric emptying. To investigate whether different stressors activate nesfatin-1 immunoreactive (IR) brain neurons male Sprague-Dawley rats (n=3/group) were subjected to 30 min of restraint or abdominal surgery (laparotomy and 1 min cecal manipulation under isoflurane anesthesia). Brains were processed for immunohistochemistry 90 or 120 min respectively after the start of the stress. Double labeling for Fos and nesfatin-1 was performed. Restraint increased the number of Fos positive neurons in the supraoptic nucleus (SO), anterior parvocellular PVN (apPVN), medial magnocellular PVN (mmPVN), lateral magnocellular PVN (lmPVN), dorsal cap of PVN (dcPVN) and locus coeruleus (LC) and of these cells 90% in the SO, 45% in the apPVN, 28% in the mmPVN, 17% in the lmPVN, 16% in the dcPVN and 69% in the LC were nesfatin-1-IR. Abdominal surgery also increased Fos expression in the SO, apPVN, mmPVN, lmPVN, and in addition in the medial parvocellular PVN (mpPVN), Edinger-Westphal nucleus (EW), rostral raphe pallidus (rRPa) and ventrolateral medulla (VLM), whereas no differences could be detected in other nuclei. Among the activated neurons, 99% were nesfatin-1-IR in the SO, 63% in apPVN, 30% in the mmPVN, 43% in the lmPVN, 7% in the mpPVN, 65% in the EW, 70% in the rRPa and 54% in the VLM. In conclusion, restraint stress and abdominal surgery activate a large proportion of nesfatin-1-IR neurons in various autonomic brain nuclei suggesting a possible role for nesfatin-1 in psychological or physical stress-related visceral responses.

5.

Gene Expression Deregulation in Response to Stress in Non-Human Primates

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Gene functions and inter-individual variation in genetic regulation related to stress response can be studied on gene transcript level. Microarray profiles of peripheral gene expression differentiate patients with a variety of psychiatric disorders and healthy subjects, suggesting an association between peripheral transcript levels and environment-triggered psychopathology. We have used genome-wide gene expression profiling in blood to elucidate, in a model system, molecular processes involved in response to a significant life stressor.

The use of a model system – in this case vervet monkeys (*Chlorocebus aethiops sabaues*) from the Vervet Research Colony – facilitates control of the variable effects of environmental influences on stress responses, in a way that is impossible in human populations. Specifically, we investigated gene expression dysregulation in vervet monkeys in response to the relocation from UCLA to Wake Forest University (North Carolina). We assumed that this life change represents a controlled and relatively uniform major stressor and therefore collected samples to permit evaluation of expression before and after exposure to the relocation stress (RS).

We assayed baseline (pre-RS exposure) and post-RS exposure genome wide transcript levels using the HumanRef-8 v2 chip (Illumina) representing more than 18K human genes. To identify genes showing differential expression in response to RS, we compared both conditions in 22 monkeys. Among 1673 probes that showed differential expression at a level of p-value < 0.05 (paired T-Test, Benjamini-Hochberg corrected) between pre- and post-RS time points, 223 genes showed a fold change of 1.2 or more. According to the Panther ontology, this group showed significant under representation of genes involved in lipid, fatty acid and steroid metabolism, as well as receptors and signaling molecules (p-value < 0.04, Bonferroni corrected). 56 and 167 probes were, respectively, up- and down-regulated. Up-regulated genes were enriched in genes involved in intracellular protein trafficking, while list of down-regulated genes showed under representation of receptor and signaling molecule genes (p-value < 0.02, Bonferroni corrected). 16 of the deregulated genes were previously associated with human disorders, in particular immune, cancer and psychiatric disorders.

6.

A Role Stress-Induced Intestinal Barrier Dysfunction in Mediating Tryptophan Catabolism-Driven Immunosuppression and Depression in BALB/c Mice

C Kiank^{1,3}, A Starke¹, S Voß¹, J-P Zeden¹, NT Nguyen¹, N Mähler¹, G Domanska¹, G Fusch², C Schütt¹

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Long-term psychological stress such as anxiety, social isolation or insecurity has powerful effects on health. If individuals are repeatedly stressed or confronted with chronic stressors they become more vulnerable to a wide range of illnesses including infections, tumors, cardiovascular diseases or depression. However, the underlying mechanisms by which stress renders an organism susceptible for such diseases remained elusive.

Here, we demonstrate in BALB/c mice that a single acute psychological stress caused “brain-gut-axis”-dependent intestinal barrier dysfunction with subsequent bacterial translocation which induced transcription of the tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) in various tissues including the brain, spleen, liver and lung. After such a single acute stress cycle homeostasis becomes rapidly reconstituted as shown by the normalization of intestinal barrier function, the tryptophan catabolism as well as stress hormone levels within 24 h after the termination of stress exposure. However, prolonged stressful periods cause an systemic immunosuppression as shown by the anti-inflammatory cytokine bias with a heightened *ex vivo* inducibility of IL10 while there is a reduced release of TNF after LPS stimulation of splenocytes, and a defective antibacterial defence measured by increased bacterial load in blood and liver 24h after experimental infection with *E. coli* which as performed immediately after the last chronic stress session. In addition, chronic stress exposure is followed by a hypercatabolic syndrome measured by a loss of body weight accompanied with hypercortisolism, hyperleptinemia and hypothyroidism, the induction of gluconeogenesis, hypotriglyceridemia but hypercholesteremia and a wasting of essential amino acids. Lastly, with increasing numbers of stress cycles we observed the development of depression-like behavior which was characterized by reduced exploration behavior, the loss of social interactions and increased huddling. Importantly, immunosuppression, loss of body mass and behavioral alterations were reduced by the treatment with an IDO-inhibitor.

Thus, enhanced catabolism of tryptophan is an essential mediator of chronic psychological stress-induced immunosuppression, metabolic alterations and depression in mice.

7.

Evidence for a Major Involvement of the Peripheral CRF₁ Signaling Pathway in the Development of Visceral Hypersensitivity in Rats

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¹*CURE: Digestive Diseases Research Center, Center for Neurovisceral Sciences and Women's Health, Department of Medicine, Division of Digestive Diseases, David Geffen School of Medicine at UCLA, Los Angeles, CA;* ²*VA Greater Los Angeles Healthcare System, Los Angeles, CA;* ³*Clayton Foundation Laboratories for Peptide Biology, Salk Institute, La Jolla, CA*

Experimental and clinical data suggest a key role of corticotropin releasing factor (CRF) receptor 1 (CRF₁) in mediating stress-induced visceral hyperalgesia. It is generally assumed that stress-related gut function responses primarily involve brain CRF₁, however recent studies also point to the possible involvement of peripheral CRF₁ signaling pathways. We recently showed that intraperitoneal (IP) injection of the selective CRF₁ agonist, cortagine, induces visceral hypersensitivity to colorectal distension (CRD) in rats and aimed to delineate the involvement of peripheral vs central CRF₁ signaling pathways in cortagine in this effect. We assessed the visceromotor response (VMR) to CRD of male SD rats (250-275g) following IP cortagine (10 µg/kg) after pretreatment with or without the CRF receptor antagonist astressin injected intracerebroventricularly (ICV) (3 or 10 µg/rat, 7 µl, i.e.= 10 or 33 µg/kg, respectively) or IP (10 µg/kg, 0.2 ml). Peripheral cortagine increased the VMR to CRD by 66.6% and 51.9% compared to baseline for the pressures of 40 and 60 mmHg, respectively. The cortagine-induced increased VMR responses at 40 and 60 mmHg were partially reduced by 70.9% and 49.0% respectively following astressin 10 µg/rat ICV 5 min before cortagine administration (Δ EMG response over baseline: 61.7 ± 42.9 and 66.5 ± 42.5 vs. astressin: 17.9 ± 18.5 and 33.9 ± 27.6 , $P > 0.05$, $n = 8-22$, for 40 and 60 mmHg respectively) while pretreatment with the lower dose of astressin (3 µg/rat) did not have any effect. In contrast, IP astressin (10 µg/kg) injected 15 min before IP cortagine abolished the VMR at both 40 and 60 mmHg (Δ EMG response over baseline: 129.6 ± 49.9 and 126.3 ± 51.0 vs. astressin: 15.7 ± 23.5 and -4.9 ± 16.3 , $P < 0.05$, $n = 7-14$, respectively). In conclusion, astressin injected ICV is less potent than IP in antagonizing IP cortagine-induced visceral hypersensitivity in rats, as shown by the 3-fold shift in the antagonist-agonist ratio. The data shows that IP cortagine selectively targets CRF₁ receptors present in the periphery to induce visceral sensitization and provides further support to the relevance of peripheral CRF₁ signaling in the development of visceral hypersensitivity.

8.

Fear, Corticosterone and Jet Lag

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Disruption of circadian timing by rapidly changing the phasing of the LD cycle to which the organism is exposed acutely impairs performance in humans and other organisms. These rapid changes mimic the effects of travel between time zones and can be referred to as experimental "jet lag." These jet lag protocols have been shown to produce desynchronization within the central circadian pacemaker, and thus produce a transitory but fundamental disruption in the

circadian timing system. We have found that jet-lag dramatically impairs the ability of mice to recall fear conditioned behavior. We propose that this impairment may, in part, be due to altered patterns of corticosterone secretion, and will present some of our findings on the interaction between corticosterone and fear conditioned behavior.

9. Cannabinoid CB1 Receptor Facilitation of Substance P Release from Primary Afferent Terminals in the Rat Spinal Cord

G Zhang, L Lao, JC Marvizón

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Substance P and its receptor, the neurokinin 1 receptor (NK1R), play a critical role in the induction of chronic pain. The main source of substance P in the spinal cord is primary afferent terminals. Substance P release from these terminals is modulated by GABA_B, mu-opioid, alpha-2 adrenergic and NMDA receptors. Here we report the existence of a novel facilitation of substance P release by cannabinoid CB1 receptors. Substance P release was evoked by stimulating the dorsal root of rat spinal cord slices, and measured in situ with NK1R internalization.

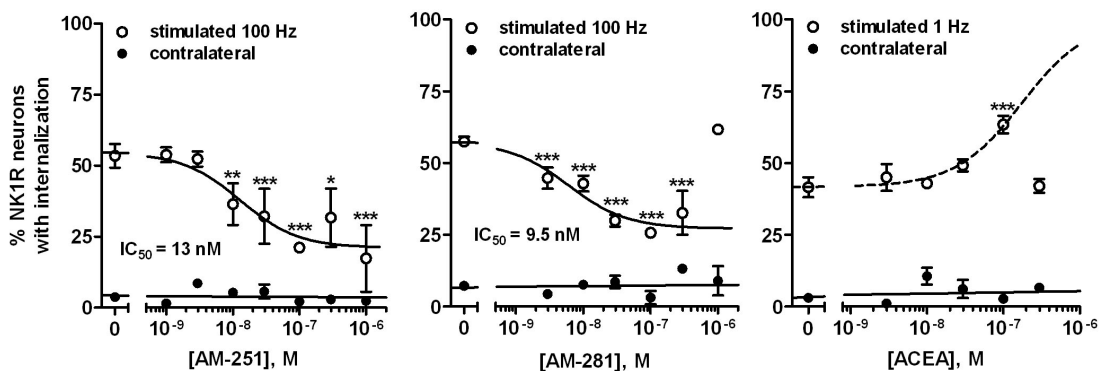
1 - NK1R internalization evoked by high frequency (100 Hz) electrical stimulation of the dorsal root was inhibited by the selective CB1 receptor antagonists AM-251 and AM-281 (Fig.). The CB1 agonist ACEA (100 nM) reversed the inhibition produced by 100 nM AM-251.

2 - NK1R internalization evoked by low frequency (1 Hz) dorsal root stimulation was inhibited by AM-251 (100 nM) and increased by ACEA (Fig.). The effects of AM-251 and ACEA cancelled each other when co-administered.

3 - NK1R internalization evoked by immersing the dorsal root in capsaicin was also inhibited by AM-251 (1 μM), and this effect was reversed by ACEA (30 nM).

4 - NK1R internalization induced by incubating the slices with 1 μM substance P was not affected by AM-251, AM-281 or ACEA (100 nM). Therefore, the effect of these compounds was on substance P release and not on NK1R internalization itself.

5 - CB1 receptors may facilitate substance P release by inhibiting GABA release from dorsal horn neurons, thus suppressing the inhibition of substance P release by GABA_B receptors. Indeed, the GABA_B antagonist CGP-55845 (100 nM) reversed the inhibition of NK1R internalization by AM-251 (100 nM).



The robust effect of the CB1 antagonists and the limited effect of the CB1 agonist suggest that endocannabinoids are released by the stimuli used, or tonically. Although cannabinoids produce analgesia, probably acting through CB2 receptors, this facilitation of substance P release by CB1 receptors suggest that endocannabinoids may also contribute to the induction of chronic pain.

10.

Short-Term Inflammation Induces While Long-Term Inflammation Decreases Segmental Opioid Release in the Spinal Cord

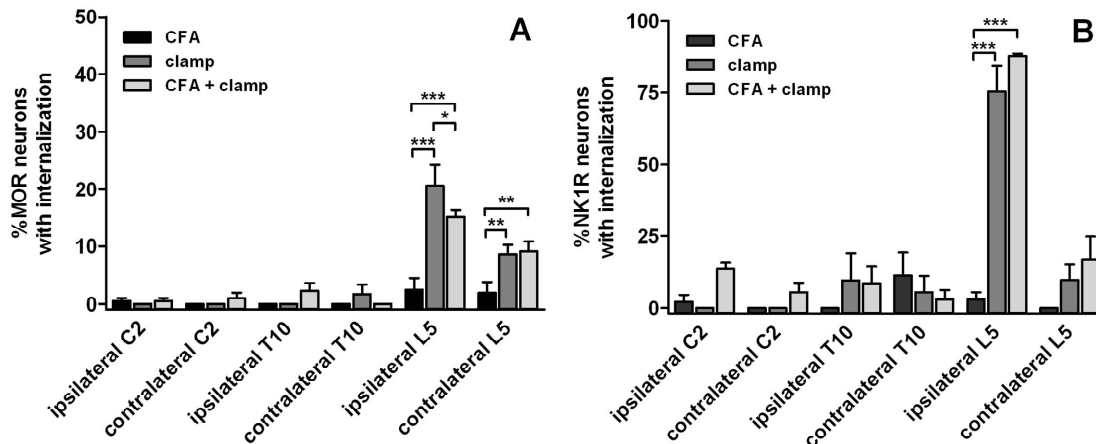
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Mu-opioid receptor (MOR) internalization can be used as an in situ measure of opioid release. We have shown (Lao et al., 2008, Brain Res. 1197:85-93) that hindpaw clamp after intrathecal peptidase inhibitors induces MOR internalization only in the spinal segments innervated by the injured paw, indicating the occurrence of segmental opioid release. In this study we measured spinal opioid release during short-term (formalin) and long-term (CFA) inflammation. Our approach was to inject peptidase inhibitors (amastatin, captopril and phosphoramidon) intrathecally, induce a pain state, and measure MOR and NK1R internalization in segments C2, T10 and L5.

Formalin (2.5%) was injected in one hindpaw of awake rats (n=4) 5 min after intrathecal peptidase inhibitors; the rats were fixed 20 min later. NK1R internalization was abundant (83±6%) in ipsilateral L5. MOR internalization was detected in both ipsilateral L5 (24±4%) and contralateral L5 (19±4%), where it was significantly higher than in saline-injected controls ($p < 0.01$). MOR internalization was negligible in the C2 and T10 spinal segments, independently of the site of the injection of peptidase inhibitors (T1 or L6). Therefore, short-term inflammation produces segmental opioid release in the spinal cord, which occurs both ipsilaterally and contralaterally.

CFA (150 μ l) was injected in one hindpaw and rats (n=4) were fixed two days later, 10 min after receiving intrathecal peptidase inhibitors. NK1R and MOR internalization were negligible in all segments, indicating that long-term inflammation does not produce sustained release of substance P or opioids. To determine whether long-term inflammation affects opioid release induced by acute pain, we compared MOR internalization induced by clamping a normal or a CFA-inflamed hindpaw. NK1R internalization induced by clamp was not affected by CFA. MOR internalization induced by hindpaw clamp in ipsilateral L5 decreased after CFA inflammation. Therefore, spinal opioid release induced by acute pain decreases during long-term inflammation.



MOR and NK1R internalization after CFA injection in the paw. CFA: subcutaneous injection of 150 μ l CFA into the plantar surface of the left hindpaw, followed two days later by intrathecal peptidase inhibitors. Clamp: intrathecal peptidase inhibitors, followed 5 min later by hemostat clamping of the left hindpaw for 30 sec. CFA + clamp: CFA injected into the left hindpaw, followed two days later by intrathecal peptidase inhibitors, 5 min later the inflamed left hindpaw was clamped for 30 sec. Rats ($n=4$ per group) were fixed 10-15 min after the intrathecal injection. MOR internalization (A) and NK1R internalization (B) in laminae I-II of the segments indicated. Two-way ANOVA: $p<0.0001$ for the variables 'treatment' and 'spinal segment' and their interaction. Bonferroni's post-test: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

11.

Chronic Stress in a Rat Model of Temporal Lobe Epilepsy: A Mechanism of Co-Morbidity Between Epilepsy and Depression

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Purpose: Post-status epilepticus (SE) epilepsy in rats is accompanied by behavioral and biochemical symptoms of depression (Mazarati et al. Brain 2008;131:2071-2083). Considering that chronic stress, and particularly the hyperactivity of the hypothalamo-pituitary-adrenocortical (HPA) axis represents an important mechanism of major depression, we examined whether such endocrine impairment contributes to the epilepsy-associated depression.

Methods: Plasma corticosterone radioimmunoassay was performed before and 8-10 weeks after pilocarpine-induced SE in Wistar rats, under basal conditions, and following dexamethasone (0.03 mg/kg) / corticotropine releasing hormone (CRH, 50 ng/kg) challenge. At the time of blood collection, animals had been seizure-free for at least 6 hours. Separate group underwent one week-long infusion of a glucocorticoid blocker mifepristone (50 nmoles) into dorsal raphe (a major source of hippocampal serotonin, and a neuroanatomical substrate of depression). Depressive behavior was examined by measuring immobility time in the forced swim test, and serotonin release from the hippocampus – by fast cyclic voltammetry.

Results: In controls, dexamethasone induced three-fold decrease, and CRH - three-fold increase of corticosterone versus basal level ($p<0.05$). In epileptic rats, basal corticosterone concentration was 30% higher versus controls ($p<0.05$). Dexamethasone was ineffective; CRH induced seven-fold increase of corticosterone ($p<0.01$ versus controls). The endocrine impairments positively correlated with the increased immobility time, and decreased serotonin release. Mifepristone reversed behavioral and biochemical correlates of depression in epileptic rats, but produced no effects in control subjects.

Conclusion: Chronic epilepsy leads to the interictal hyperactivity of the HPA axis, which may induce depression via compromising raphe-hippocampal serotonergic transmission.

Supported by NIH grant NS046516.

12.

Decreased Behavioral Pain Responses During Phase 2 of the Formalin Test in Mice with Selective Knockdown of NMDA Receptors in Primary Afferent Neurons

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Aim of Investigation: The role of NMDA receptors (NMDARs) expressed on spinal afferent neurons in nociceptive transmission remains controversial. The aim of this project was to develop mice with tissue selective knockdown of NMDARs on these neurons and evaluate their behavioral responses to different kinds of painful stimuli.

Methods: Mice engineered with loxP sites flanking exons 11-22 of the NMDAR NR1 subunit gene (floxed-NR1) were crossed with mice engineered to express Cre recombinase under the control of the peripherin promoter (Prph-Cre). Male peripherin-Cre⁺ floxed NR1 mice were compared to Cre⁻ floxed NR1 mice littermates. Western blotting and immunocytochemistry were used to evaluate expression and localization of NR1. FURA-2 imaging of [Ca²⁺]_i responses was used to evaluate functional responses to NMDA in cultured DRG neurons. Nociception was evaluated using the hotplate, tail withdrawal, tail pressure and formalin tests.

Results: Western blotting of whole cell extracts of DRG indicated a 73 ± 7% reduction in NR1 expression (p<0.001) with no effect on NR1 expression in spinal cord or brain. Immunocytochemistry with antibodies to NR1 in sections of DRG revealed a 75 ± 7% overall decrease in staining which was nearly uniform across all size classes of DRG neurons. A portion (17 of 30) of bradykinin-responsive DRG neurons from Cre⁻ mice responded to NMDA with an increase in [Ca²⁺]_i, while only 3 of 19 neurons from Cre⁺ mice responded. There was no significant difference between Cre⁺ and Cre⁻ mice in response latencies to the hotplate or tail withdrawal tests of thermal nociception, nor was there a difference in responses to tail pressure. However, compared to Cre⁻ littermates, Cre⁺ knockdown mice had a 50% decrease in the phase 2 response to injection of formalin solution (p<0.05). There was no effect on phase 1 responses.

Conclusions: Tissue specific knock down of NMDARs using peripherin promoter driven Cre expression results in a selective ~75% decrease in NR1 expression in DRG neurons. The decrease in phase 2 of the formalin test suggests important role NMDA receptors on primary afferent nerves in inflammatory hyperalgesia.

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13.

Characterization of a New Mouse Model for the Study of Orofacial Pain

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Multiple highly prevalent and debilitating conditions involve pain in the trigeminal distribution (head, face, masticatory musculature, temporomandibular joint and associated structures). Although effective treatments for trigeminally mediated pain exist, there are a large number of patients for whom these treatments are either ineffective or poorly tolerated. The understanding of the pathophysiology of pain in the orofacial region remains incomplete, and there are very few models that focus on this problem. Advances in transgenic mouse technology have led to multiple new tools for the study of pain. The aim of our study is to develop a mouse model for OFP that will enable us to take advantage of these advances. We have studied the behavioral and histological responses of C57BL/6 female mice to injection of the noxious/inflammatory agent Complete Freund's Adjuvant (CFA) into the masseter muscle. 8 animals in each group received either CFA or saline control solution into the right masseter muscle. The grooming patterns after the injection were assessed with continuous video monitoring. Mice injected with CFA exhibited four different behaviors: Intense facial grooming (face rubbing, bilateral); chin rubbing (ipsilateral); face scratching (ipsilateral) as well as enhanced chewing in comparison with controls. 24hrs after the injection, mice were perfused and brain slices were processed for c-fos staining as well as for glial activation (GFAP and CD45). Expression of c-fos neurons in the trigeminal nucleus caudalis (TNC) of the CFA group were significantly higher versus control. The number of activated astrocytes (GFAP) as well as microglia (CD45) was also significantly higher in the CFA group versus control. Other areas mapped expressing c-fos in addition to TNC were the nucleus oralis, interpolaris, inferior salivatory nucleus, areas of facial nucleus, areas of noradrenaline and adrenaline cells, solitary nucleus area, rostroventral reticular nucleus, lateral reticular nucleus, and nucleus ambiguus. In conclusion, mice show novel behaviors and histological activation in trigeminal pain structures with our orofacial pain model. We can use this model to advance our understanding of the mechanisms of trigeminal pain, and as a platform to identify new therapeutic approaches.

14.

Increased Excitability in the Mouse Hippocampus during Pregnancy

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Neurosteroid levels become highly elevated during pregnancy inducing plasticity of GABA_A receptors (GABA_ARs). These plastic changes are most likely homeostatic and evolutionary adaptations to allow the mammalian brain to cope with increased neurosteroid levels. Preliminary data from Western blot analysis reveals a downregulation of GABA_AR δ -subunit expression in the hippocampus and midbrain during pregnancy, which reverts to baseline levels postpartum. On the other hand, there is no change in δ -subunit expression levels in the frontal cortex, indicating that the alterations in GABA_ARs are brain region specific. Field recordings

from hippocampal slices from pregnant mice show an increased excitability compared to virgin mice as evidenced by a shift to the left of the stimulus vs. slope of the fEPSP curves. Furthermore, a higher percentage of slices from pregnant mice show epileptic discharges in presence of high K^+ . This increased hippocampal excitability can be reduced by perfusing the progesterone metabolite neurosteroid allopregnanolone (ALLO) – at a concentration (100 nM) that is in the range found during pregnancy. Field recordings from *Gabrd*^{-/-} mice, which lack GABA_AR δ -subunits altogether and thus are devoid of alterations in δ -subunit levels during pregnancy, do not reveal any increased excitability in the hippocampus during pregnancy, although the epileptiform activity in the presence of high K^+ is more severe and longer lasting compared to WT littermates. In the cortex, where there are no changes in δ -subunit levels during pregnancy, field recordings show no signs of increased excitability in pregnant female mice. Altogether these results indicate that the increased excitability in the hippocampus during pregnancy is probably due to the downregulation of the δ -subunit. However, physiologically increased levels of ALLO during pregnancy restore inhibition to a level similar to that in virgin animals. Consistent with this hypothesis we don't see any differences in excitability in a genetic mouse model of epilepsy (Synapsin1 knock out mice) during pregnancy.

15.

The Oligosomatostatin Receptor Agonist ODT8-SST Increases Food Intake While Decreasing Body Weight in Rats

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Background: Somatostatin (SST) and octreotide were shown to affect food intake and are recently recognized as potential targets in the drug treatment of obesity. SST exerts its biological action by interacting with 5 SST receptors (SST-R) although the subtype(s) involved in modulating food intake are unknown.

Aim: To investigate the central action of SST analogs with differential SST-R affinities on food consumption in rats.

Methods: Freely fed male rats (280-350g) implanted with a chronic intracerebroventricular (ICV) cannula were injected with des-AA^{1,2,4,5,12,13}-[D-Trp⁸]SST, ODT8-SST (SST-R affinity: 4>3>1>2=5) and selective peptide SST2 or SST4 agonists during the light phase. Food intake (g/300g body wt) and body weight were monitored for 24 h. Solid food gastric emptying (GE) was investigated in 20-h fasted rats. Neuronal activity was assessed by Fos immunohistochemistry 90 min after ODT8-SS ICV.

Results: ODT8-SST (1 μ g/rat, ICV) significantly increased food intake during the 1st h (4-fold), 2nd h (6-fold), and 2-4 h ($p=0.01$) whereas the 24 h cumulative food intake was not altered. The body weight 24 h post injection was decreased (mean \pm SEM: -8.0 ± 2.2 g vs. 0.3 ± 1.2 g, $p=0.003$). The selective SST4 receptor agonist (1 and 3 μ g/rat, ICV) did not modulate food intake and body weight during 24 h whereas a selective SST2 receptor agonist (1 μ g/rat, ICV) also increased the 9 h cumulative food intake compared to vehicle (9.08 ± 1.59 vs. 1.38 ± 0.43 , $p<0.001$) followed by a significant reduction of dark phase food intake (10.60 ± 1.31 vs. 20.94 ± 1.02 , $p<0.001$) without significant effect on 24 h body weight. In contrast to ICV, ODT8-SST intraperitoneally (IP, 30 μ g/kg) did not alter the 2 or 4 h food intake while reducing the 24 h body

weight compared to vehicle (-0.4 ± 0.4 g vs. 2.3 ± 0.9 g, $p=0.012$). ODT8-SST ($1 \mu\text{g}/\text{rat}$, ICV) increased the 2 h GE compared to vehicle ($85.9 \pm 4.6\%$ vs. $66.5 \pm 5.8\%$, $p<0.05$), whereas IP had no effect. ODT8-SST ($1 \mu\text{g}/\text{rat}$, ICV) increased the number of Fos positive neurons selectively in the supraoptic nucleus (SON, 131.0 ± 23.8 vs. 0.8 ± 0.1 , $p=0.006$).

Conclusions: ICV ODT8-SST exerts a long-lasting orexigenic effect associated with a 24 h body weight reduction suggesting an increase in energy expenditure. Activation of SST2-R and SON may contribute in part to ODT8-SST alterations of food intake and energy homeostasis.

16.

Peripheral Urocortin 2 Stimulates Release of Colonic Nitric Oxide and Inhibits Colonic Contractions In Vivo in the Rat

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Background: Corticotropin-releasing factor (CRF) related peptides and their receptors (CRF₁ and CRF₂) play role in the gut secretomotor response to stress. CRF-peptides stimulate the colon by activating CRF₁ receptors. The role and mechanisms of CRF₂ mediated effects on the colon are not known.

Aims: Determine the effects of selective CRF₂ activation on colonic motor response and nitric oxide production in the rat.

Methods: Urethane anesthetized (1.5 g/kg ip) male SD rats were fitted with rod-shaped sensors in the transverse colon submucosal space (~ 8 cm from the anus) for electrochemical detection of NO and with mini-pressure transducer placed in the lumen of the transverse-colon, through the anus, for colonic contraction measurement. CRF₂ and neuronal NOS (nNOS) colocalization in the colonic submucosal and myenteric plexi neurons was determined.

Results: The selective CRF₂ agonist, Ucn 2, induced NO production in a dose-dependent (1 - $10 \mu\text{g}/\text{kg}$, iv). Ucn 2 ($10 \mu\text{g}/\text{kg}$, iv) raised colonic NO concentration (basal: 19.4 ± 5.6 nM to 34.1 ± 8.3 nM, $p<0.05$) and reduced colonic contractions ($-30 \pm 9.5\%$ from baseline; $p<0.05$). L-NAME (10 mg/kg, iv) rapidly decreased the Ucn 2-induced NO elevation to basal level whereas S-nitro-N-acetyl-penicillamine (1 mg/kg, iv), a NO donor, increased colonic NO level (19.4 ± 5.6 vs 27.9 ± 9.2 nM, $p<0.05$). By contrast, a selective CRF₁ agonist, cortagine ($10 \mu\text{g}/\text{kg}$, iv) did not affect colonic NO level or colonic contractions. The selective CRF₂ receptor antagonist, astressin₂-B ($50 \mu\text{g}/\text{kg}$, iv) prevented Ucn 2 ($10 \mu\text{g}/\text{kg}$)-induced colonic NO elevation (control; $109.8 \pm 3.2\%$, astressin₂-B; $19.3 \pm 4.1\%$ of the peak response to Ucn 2 alone, $p<0.01$) and the inhibition of colonic contractions (control; $-26.2 \pm 18.0\%$, astressin₂-B; $+18.3 \pm 8.3\%$ from baseline; $p<0.05$). CRF₂ is colocalized with nNOS in the neuronal cell body and fibers of colonic submucosal and myenteric plexi.

Conclusion: Simultaneous detection of colonic NO production and contraction in vivo revealed that selective CRF₂ activation, stimulates NO production and inhibits transverse colonic contractility in rats. Co-localization of CRF₂ and nNOS in the colonic enteric neurons suggests that these neurons could be a peripheral site of action for Ucn 2. The link between CRF₂ signaling and NO generation in the rat colon may have relevance in the coping response of the colon to stress.

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17.

Sympathetic Over-Activation by Brainstem Thyrotropin-Releasing Hormone Contributes to Cardiovascular Mortality in Type 2 Diabetic Goto-Kakizaki Rats

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Background: Cardiovascular disease is the leading cause of mortality in type 2 diabetic (T2D) patients. Brainstem thyrotropin-releasing hormone (TRH) synthesized in raphe nuclei and acting in the rostroventrolateral reticular nucleus to activate sympathetic pathways may contribute to spontaneous or obesity-induced hypertension. T2D Goto-Kakizaki (GK) rats have increased brainstem TRH receptor expression and sympathetic over-activation after intracisternal injection (ic) of TRH analog, RX 77368 (RX).

Aim: To test the hypothesis that sympathetic over-activation by brainstem TRH contributes to the hypertension, tachycardia, and increased cardiovascular mortality in T2D.

Methods: Blood pressure (BP) and heart rate (HR) were measured by tail-cuff in basal and every 30 min for 120 min after ic RX (under 2 min isoflurane anesthesia) in conscious male non-diabetic Wistar and T2D GK rats. Some GK rats were pretreated with autonomic receptor blockers, an angiotensin converting enzyme inhibitor captopril, or angiotensin type 1 receptor (AT1) blocker irbesartan.

Results: RX (10-60 ng) ic dose-dependently increased BP. GK rats showed basal hypertension (152±2 vs 124±3 mmHg in Wistar rats) and significantly potentiated BP responses to ic RX (236 mmHg) vs Wistar rats (168 mmHg). Basal HR was the same in the two strains. HR did not change after ic RX in Wistar rats but increased significantly in GK rats (+88/min vs basal). A dose-related mortality within 120 min was shown only in GK rats (20 ng 25%, 30-60 ng 100%) that was associated with an extreme hyperglycemia (500-600 mg%). In GK rats, hexamethonium (10 mg/kg) blocked ic RX 30 ng-induced increase in BP/HR and reduced the mortality (14%). Phentolamine (2 mg/kg) totally prevented the hypertensive response while enhanced the increase in HR (+160) and reduced the mortality by 50%. Bretylium (25 mg/kg) did not influence the effects of ic RX on BP and HR but shortened the rat survive time (100% died before 60 min). Captopril (500 mg/L in drinking water for 8 days) lowered basal BP (131±8 mmHg) but significantly enhanced ic RX 20 ng-induced hypertension (224 vs 172 mmHg) and tachycardia (432 vs 367/min) with a 83% mortality. Angiotensin type 1 receptor blocker irbesartan prevented the increases in BP/HR and mortality. mRNAs of the indicators of oxidative stress and inflammation in the heart, plasma indicators of oxidative stress, and triglycerides increased by ic TRH analog only in the T2D GK rats while plasma cholesterol decreased only in Wistar rats.

Conclusion: 1) Cardiovascular mortality is induced in T2D GK rats by activating brainstem TRH receptors; 2) potentiated hypertension and tachycardia resulted from sympathetic over-activation by TRH is responsible for the increased mortality; (3) peripheral catecholaminergic alpha receptor mediates the hypertensive response; (4) increased heart and plasma oxidative stress may participate the cardiovascular responses to ic TRH analog in T2D GK rats; (5) the AT1 receptor mediates or modifies these responses; (5) inhibiting angiotensin converting enzyme results in enhanced BP/HR responses to ic RX that may be mediated by a compensative increase in peripheral catecholaminergic receptors;

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18.

Corticotropin-Releasing Factor Binding Protein (CRF-BP) in the Rat Brainstem Autonomic Nuclei and Colon: Expression, Localization and Regulation by Stress-Induced Stimulation in Colonic Motility

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Background: Activation of central or peripheral CRF₁ receptor by CRF or urocortin 1 (Ucn 1) stimulates colonic function. CRF-BP binds the CRF₁ agonists, CRF and Ucn 1 with a greater affinity than the CRF receptors limiting peptide availability for CRF receptor mediated actions in the forebrain (PNAS 98:11142-47, 2001).

Aims: To establish the gene expression and cellular location of CRF-BP in brainstem autonomic nuclei and colon, and regulation of colonic CRF-BP by stress-induced stimulation of colonic motor function in rats.

Methods: Tissue samples were collected from 3 adult male SD rats (282-304g). The area postrema (AP), nucleus tractus solitarii (NTS), dorsal motor nucleus of the vagus (DMN), locus coeruleus (LC) and barrington nucleus (Bar) were captured using laser capture microdissection system (LCM) from the brainstem sections (8 µm). Proximal colon was separated into mucosa and submucosa plus muscle layers. RT-PCR was performed using primers for rat CRF-BP. The cryostat sections of cortex, brainstem (25 µm) and proximal colon (5 µm), and whole mount preparation of colonic enteric plexus were processed for CRF-BP immunohistochemistry and double staining of CRF-BP/Hu, a neuron marker. Adult male SD rats (5-8/group) were treated with lipopolysaccharide (LPS, 100 µg/kg BW, ip), interleukin 1β (IL-1β, 1.5 µg/kg, iv) or water avoidance stress (WAS, 60 min). Proximal colon was collected 6 h after injections or 60 min after WAS. RT-PCR was performed and PCR products were quantitatively analyzed using Scion imaging software. The 60-min fecal pellet output (FPO) was monitored.

Results: Transcript of CRF-BP was detected in Bar>DMN>NTS>LC>AP and in the colon with the mucosa having a 4.3-fold higher level than in submucosa plus muscle layers. CRF-BP immunoreactivity (IR) was detected in the neurons of AP, NTS, DMN, LC, Bar as in those of central amygdala (positive control). In the colon, CRF-BP IR was mainly localized in crypt and lamina propria cells, and co-localized with Hu C/D in enteric plexus. CRF-BP mRNA in the colon was down-regulated (p<0.05) by ip LPS (23±4 vs saline:100±13), iv IL-1β (58±10 vs saline:100±8) and WAS (65±7 vs non-stress:100±13). All stressors significantly increased FPO (p<0.05).

Conclusions: CRF-BP is expressed in brainstem nuclei regulating autonomic outflow to the viscera and in colonic mucosa and myenteric neurons. The robust down regulation of colonic CRF-BP by immune and psychological stressors that increase colonic motor function, suggests that CRF-BP may play a local modulatory role by increasing the levels of “free” colonic CRF and Ucn 1 that participate in stress-induced stimulation of colonic motility.

19A.

Hippocampal Contribution to Renewal of Conditional Fear After Extinction

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The tenacious nature of conditional fear has long been established. Although fear responding can be extinguished through repeated presentations of a feared stimulus (CS), a number of phenomena reveal that the original fear memory remains intact. In *renewal*, a presentation of the CS outside of the extinction context causes fear to return (e.g., Bouton & Bolles, 1979a). The context-sensitive nature of renewal makes the involvement of the hippocampus (HPC) a prime target for investigation, as the HPC has been shown to be responsible for the encoding and retrieval of contextual information (Fanselow, 2000). Recent findings have delivered conflicting results: Frohardt et al. (2000) concluded that the HPC is not required for renewal, whereas Ji & Maren (2005) concluded that it is. These studies differ in how they test for and analyze fear. In the former, responding during the first 30-second CS was analyzed. In the latter, average responding to a continuous tone was analyzed. The present study sought to resolve this discrepancy. Rats received either sham or excitotoxic lesions of the dorsal hippocampus (DH). Subsequent training consisted of tone-shock pairings, followed by two days of extinction. In the final phase, animals were brought back to the original training context to test for fear renewal with either 4 tones or a continuous tone (controls were tested in their extinction context). During the *initial 30 seconds*, freezing in the renewal group was significantly greater than freezing in the controls, revealing a classic renewal effect, with no significant effect of lesion type. However, data from the *average* percent freezing revealed a significant decrease in freezing in the lesioned renewal animals when compared to the sham renewal animals. In other words, deficits in fear renewal slowly emerged over the course of testing, regardless of the test condition. These results show that the “discrepancy” over the role of the DH in renewal is actually dependent on differences regarding *when* the DH is involved. This suggests that renewal is comprised of more than one mechanism: a DH-dependent mechanism responsible for *average* fear responding and a DH-independent mechanism responsible for *initial* levels of fear. In order to test this idea, we questioned whether the infralimbic (IL) region of the prefrontal cortex could be responsible for initial levels of fear. Using the same protocol but with IL lesions, we found no attenuation of renewal. However, IL lesions enhanced spontaneous recovery in *initial* (not *average*) moments of fear. Taken together, these experiments suggest that recovery of fear seems to involve a complicated network of structures that operate in a unique and time-specific manner.

19B.

GABA_AR Plasticity During Pregnancy and the Pathological Consequences of Its Dysregulation in the Postpartum Period

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The δ subunit-containing GABA_ARs are uniquely sensitive to neurosteroids. These brain-derived metabolites of steroid hormones potentiate the effects of GABA on its δ subunit-containing

receptors and also alter receptor expression. Thus, the regulation of the expression and function of GABA_AR δ subunit is of great relevance during pregnancy and postpartum, when there are robust and abrupt changes in steroid hormone levels. Here we demonstrate brain region-specific alterations in the expression of δ subunits during pregnancy, including a significant decrease in their expression levels in the dentate gyrus, striatum, and thalamus. However, there were no significant differences in the expression of δ subunits in the cortical areas of pregnant mice compared to virgin. The adaptive function of such downregulation in specific brain areas may be to maintain neuronal excitability in a constant range in the face of dramatic hormonal changes, thereby preventing abnormal behaviors during and after pregnancy. Consistent with this hypothesis, severe behavioral abnormalities are present in a mouse line genetically engineered to lack δ subunits, *Gabrd*^{-/-} mice. These mice are unable to regulate GABA_AR δ subunit expression and function during and after pregnancy. In the postpartum period such mice show deviant maternal behavior, anxiety-like and depression-like actions. The abnormal maternal behaviors are associated with a decrease in pup survival due to either cannibalization or neglect. Cross fostering experiments demonstrate that the decreased survival rate is correlated with the mother's genotype, implying that the decrease in survival is not due to an inherent defect in the pups. Behavioral tests on the F1 generation of the cross-fostered animals show that pups, regardless of their genotype, exhibit anxiety-like and depression-like behaviors when reared by *Gabrd*^{-/-} mice. Overall these data are consistent with a compensatory downregulation in the expression of the GABA_AR δ subunit during pregnancy, and with the idea that dysregulation of this plasticity in *Gabrd*^{-/-} mice results in abnormal behaviors in the postpartum period that can negatively influence pup behavior.

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Abstracts of Posters

Clinical

20.

Do IBS Symptoms Vary Between Men and Women? A Meta-Analysis

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Introduction: IBS is a female predominant disorder marked by abdominal pain and altered bowel habits. Sex differences in IBS have been reported with regard to prevalence, clinical symptoms, response to treatment, and physiology. There is also evidence for increased symptoms and enhanced visceral perception at time of menses, suggesting that female sex hormones may be a contributing factor. However, data regarding sex differences are limited and conflicting. We performed a meta-analysis to: 1) compare the prevalence of IBS symptoms between sexes, 2) examine the effect of menstrual cycle on IBS symptoms, and 3) examine the effect of menopausal status on IBS symptoms.

Methods: We performed a systematic review of MEDLINE to search for studies comparing IBS symptoms between sexes, menstrual cycle phases, and menopausal states in IBS and/or healthy individuals. We performed meta-analysis to compare the relative risk (RR) of IBS symptoms between sexes.

Results: Thirteen studies reporting sex differences in IBS symptoms. In both the general and IBS population, the RR for each cardinal symptom was higher in women than men, although the difference for some symptoms was insignificant (**Table**). Eleven studies measured IBS symptoms by menstrual cycle, and 40-60% of patients reported increased prevalence and severity of IBS symptoms during menses. Of the 3 studies evaluating the effect of menopause on GI symptoms, only nausea was more prevalent in pre- vs. post-menopausal women with IBS. In healthy women, gas and flatulence were more prevalent in the post- vs. pre-menopausal women.

Conclusion: Women are more likely to report IBS symptoms than men, particularly those associated with constipation. This may help explain the female predominance in IBS-C and chronic constipation, in particular. Female sex hormones may influence IBS symptoms, but larger studies with more accurate assessment of menstrual cycle phases are needed.

| Symptom | # studies (Overall) | RR favoring women (95% CI) | # studies (IBS only) | RR favoring women (95% CI) |
|-----------------------|---------------------|----------------------------|----------------------|----------------------------|
| Distension | 6 | 1.32 (1.14,1.51) | 4 | 1.39 (1.20,1.61) |
| Bloating | 8 | 1.16 (1.04,1.29) | 4 | 1.22 (1.05,1.42) |
| Incomplete evacuation | 10 | 1.11 (1.02,1.20) | 6 | 1.24 (1.03,1.50) |
| Infrequent stools | 4 | 1.11 (1.03,1.19) | 1 | NA |
| Abdominal pain | 5 | 1.11 (1.01,1.23) | 1 | NA |
| Lumpy/hard stools | 7 | 1.10 (0.98,1.23) | 4 | 1.24 (1.09,1.41) |
| Straining | 8 | 1.09 (1.01,1.18) | 4 | 1.02 (0.91,1.14) |

NA=Not applicable (insufficient studies).

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21.

Stress-Related Biomarkers and Symptoms Are Predictive of Irritable Bowel Syndrome (IBS)

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Introduction: IBS is currently diagnosed using symptom-based criteria, even though symptom expression is heterogeneous. Postulated biomarkers of IBS include markers of the stress response and immune system.

Aims: 1) To determine if specific biomarkers are predictive of IBS, 2) To determine if predictive biomarkers differ by sex, and 3) To determine if non-GI symptoms strengthen the association of the biomarker with IBS.

Methods: IBS was defined by Rome criteria. Biomarkers and symptoms were measured at the time of a visceral stressor (sigmoidoscopy with sigmoid colon biopsies). Biomarkers included: 1) salivary cortisol response defined as change from basal to peak levels, 2) basal plasma catecholamine levels, 3) mucosal expression of cytokines and adrenergic receptors ($\alpha_{2A-C}AR$) using real-time PCR, and 4) mast cell and lymphocyte counts. Non-GI symptoms including psychological symptoms (HAD) and quality of life (mental [MCS] and physical [PCS] components of SF-36) were measured. Univariate analyses were performed to identify biomarkers associated with IBS. Significant biomarkers were entered into a multivariable model to assess which biomarkers independently predicted IBS ($p < 0.05$). Similar analyses were performed to identify non-GI symptoms that contributed to the biomarker predictability of IBS.

Results: 45 IBS patients (26F, 19M) and 41 healthy controls (22F, 19M) were studied. In women, increased stress-induced cortisol response ($r^2 = 0.30$, $p = 0.002$) was predictive of IBS. The presence of anxiety improved the predictive strength of the model ($r^2 = 0.50$, $p < 0.001$). In men, increased mucosal expression of $\alpha_{2A}AR$ predicted IBS ($r^2 = 0.15$, $p = 0.02$). The predictability of the model improved by the addition of depression and PCS ($r^2 = 0.46$, $p < 0.001$). Overall, the only immune measure that significantly predicted IBS was mucosal IL-8 ($r^2 = 0.07$, $p = 0.03$) with lower expression associated with IBS but the contribution was small ($< 10\%$ of variance).

Conclusion: Biomarkers that predict IBS differ by sex. However, in both sexes, the strongest biomarkers are related to the two arms of the central stress response (HPA axis and SNS), with a minor role of mucosal immune markers. Non-GI symptoms such as anxiety and depression enhance the predictive strength of these biomarkers. These findings support that IBS is multidimensional, including a significant role of CNS-related factors.

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22.

Ultrasound-Guided Trigger Point Injections for Treatment of Fibromyalgia

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Myofascial pain syndrome commonly manifests in female fibromyalgia patients as a variety of pain symptoms. The UCLA Center for East-West Medicine piloted an innovative training program in trigger point technique using ultrasound technology for medical students who were enrolled in first- and fourth-year electives. The courses engaged students in a patient-focused approach to clinical problem solving that incorporated therapies from different traditions of medicine, which included trigger point injections. Myofascial pain is under-emphasized in medical education and under-recognized in clinical practice. Trigger points are diagnosed clinically, since they demonstrate no reproducible anatomic or histologic abnormalities. However, inter-rater reliability in myofascial trigger point examination has been demonstrated in the literature. Trigger point injections and dry needling of trigger points have become widely accepted for the treatment of myofascial pain syndrome. A variety of solutions may be injected, while studies widely support that the therapeutic effect in both dry needling and injection is mechanical disruption by the needle. Despite the utility of trigger point injections in medical practice, a training device is not widely used to train medical students, residents, and physicians in the technique. A total of 26 medical students in 2006 (10 first-year students and 16 fourth-year students) participated in trigger point injection workshops that incorporated ultrasound technology. The students were instructed in the clinical criteria of myofascial pain, learned how to identify trigger points, and observed ultrasound images of trigger point injections. The 26 course participants successfully completed the experiential exercise and noted the following benefits: learned how to correlate clinical information with real-time, dynamic radiological images; observed the twitch response, which is well described but not well quantified in the literature; and visualized correct placement of the needle tip to avoid iatrogenic harm such as pneumothorax. Based on these outcomes, ultrasound technology was a useful tool for training medical students in evaluation and treatment of myofascial pain. In this workshop, radiological imaging permitted in vivo visualization of hidden anatomy and offered physiologic as well as anatomic insights.

23.

Evidence for Altered Brain Circuits Underlying Selective Attention to Negative Emotional Stimuli in IBS

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Background: Hypervigilance and attentional bias towards visceral sensation has been implicated in central pain amplification in IBS.

Aim: To identify the neurobiological substrate mediating attention bias between IBS and healthy controls (Ctrls).

Methods: Brain activity in 23 female Rome+ IBS patients and 15 healthy women controls was studied using a Siemens Allegra 3T MRI scanner, using validated emotional reactivity (Emotional Matching vs. Forms Matching) and modulation (Emotional Labeling vs. Gender Labeling) paradigms that involve the viewing and labeling of negatively valenced emotional faces to activate brain circuits involved in emotion processing and arousal. Reaction Time (RT) was measured via Superlab presentation software. Paired group t-tests and a contrast analysis using a random effects general linear model were applied to test for difference in reaction time due to the interaction of group and condition. SPM5 was used to assess the relationship between reaction time and the brain activity and to test for group differences in these correlations using a false discovery rate of 5%.

Results: Both IBS and CtrlS showed significantly slowed responses during emotion matching and emotion labeling tasks compared to the respective control tasks (p 's<.001). There was an observable trend for IBS patients to have slower RTs during all conditions, however, these differences only achieved statistical significance during emotion matching and labeling (p 's<.01). Across all tasks, group differences were observed in the relationship between the RT and the medial prefrontal cortex (mPFC)/rostral anterior cingulate cortex (rACC), subgenual ACC, dorsal and lateral PFC, hippocampus, and dorsal pons. Examining the within group correlation with RTs revealed that IBS patients had more robust correlations whereas no regions could be considered significantly correlated in CtrlS. Further exploring these results by extracting the correlations (betas) from the representative mPFC voxel using the VOI toolbox in SPM revealed a moderately strong negative correlation between RT and mPFC/rACC for IBS ($r = -.38$, $p < .001$) but not for CtrlS ($r = -.07$, $p = .56$).

Conclusions: IBS patients demonstrated selective attention biases primarily towards negative emotional stimuli in comparison to CtrlS. The group differences observed in the correlation between RT and PFC regions and the inverse correlation of RT and mPFC in IBS subjects, suggests that slower RTs in IBS are associated with reduced engagement of a PFC region involved in corticolimbic inhibition.

24.

Gastrointestinal-Specific Anxiety in Inflammatory Bowel Disease: Further Validation of the Visceral Sensitivity Index

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Background: The Visceral Sensitivity Index (VSI) is the first instrument developed to assess gastrointestinal specific anxiety (GSA) including the cognitive, affective, and behavioral response to fear of GI sensations, symptoms, and the context in which these visceral sensations and symptoms occur. The VSI has demonstrated excellent psychometric properties and predictive validity in studies of symptoms and quality of life in Irritable Bowel Syndrome (IBS).

Aim: To cross-validate the use of the VSI for assessment of GSA in patients with inflammatory bowel disease (IBD).

Methods: Two clinic samples (CD $n=47$; UC $n=13$; 57% F) were administered the VSI along with measures of quality of life (the SF-12), general anxiety and depression symptoms (Hospital Anxiety and Depression Scales), disease activity scales (Harvey Bradshaw score for CD, Partial Mayo and Simple Clinical Colitis Activity Index for UC), and somatic symptom severity (PHQ-

15). Data from the initial validation sample of IBS was utilized for comparison of psychometric results.

Results: The VSI demonstrated excellent internal consistency (Cronbach's alpha = .96) and the factor analysis supported the unitary dimension of the scale (first factor = 63% of the total variance). VSI scores were moderately correlated with quality of life, anxiety, depression, and somatic symptom severity ($r = -.39, .49, .54, \text{ and } .53$ respectively, all $p < .05$). These values are similar to those found for the scale in IBS. A multiple regression model indicated the VSI was the strongest predictor of quality of life ($p < .05$) and shows a trend for it to mediate the relationship between general anxiety and quality of life ($p < .05$). Also similar to IBS, disease activity parameter scores were moderately correlated with general anxiety ($r = .27, p < .05$), and also moderately correlated with the VSI ($r = .37, p < .01$).

Conclusion: Overall, the VSI demonstrated excellent psychometric properties when administered to an IBD patient sample, providing further support for its use as an outcome assessment in clinical studies. These findings also point to the potentially important role for disease-specific anxiety in determining psychosocial outcomes in IBD.

Conclusion: This study shows no change in sexual arousal and pain sensation with the use of oral contraception despite a significant decrease in free testosterone levels in subjects using OCP's vs controls.

25.

Salivary Alpha Amylase Measured with Portable Biosensor Linked to Psychosocial Stress

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Background: A vast literature demonstrates the utility of salivary analytes to quantify stress responses, but the assay techniques employed hitherto are elaborate, often expensive, and do not allow for real-time, in-field resolution and sequencing of the biomarker across multiple assessments. A prototype hand-held biosensor that detects and quantifies salivary alpha amylase (SAA) rapidly (2 min) was tested. A specific biological indicator of adrenergic activity under conditions of stress, SAA has been shown to react and peak more quickly than other stress markers, is less susceptible to diurnal variations, and robust against sampling confounders such as saliva flow rate and contaminants (e.g., blood).

Methods: The prototype was tested in healthy males ($N=52$, aged 22-35) recruited from the pool of 420 dental students at the UCLA School of Dentistry. The biosensor was home-tested over the diurnal cycle under low stress (early in the academic session) and high stress (during final exams) and SAA values compared to conventionally collected and laboratory assayed SAA. Trait anxiety, psychological distress, and momentary subjective distress ratings (SDR) were also assessed.

Results: SDRs did not change across the day, but rose from low to high stress phases. Biosensor SAA (B-SAA) and SDRs were highly correlated under the low stress phase ($r = .48, p < .001$), and showed a trend at the high stress phase ($r = .26, p = .07$). B-SAA change scores were correlated with SDR change scores ($r = .34, p = .01$) and with psychological symptoms (e.g., test anxiety: $r = -.39, p = .01$). B-SAA rose over the day at the high stress session, but not under low stress, perhaps due to procedural novelty (naturalistic low and high stress conditions were not counterbalanced for order). Participants who scored above the median on a global severity of distress index (BSI) or trait anxiety reduced SAA levels from low to high stress sessions,

whereas those below the median retained a similar range over both sessions. Conventional collection with absorbent material did not yield accurate SAA values.

Conclusions: The biosensor prototype was easy to use, correlated with traditional measures of psychosocial stress, and was not subject to the limitations of conventional saliva collection methods. Further studies will be needed to replicate diurnal SAA values and changes under stress in high and low-anxious individuals.

26.

Psychosocial Stress Biomarker Composite Predicts CD4 Counts in Poor HIV+ African American and Latina Mothers

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Background: Specific biomarkers (e.g., cortisol and catecholamines) are theorized to reflect cumulative psychosocial stress (McEwen 2004). Studies among primarily healthy groups have shown success in using composites of these biomarkers to predict later physical and cognitive decline, reflect the presence and severity of mental health symptoms, and estimate the size of the hippocampus. The utility of a similar biomarker composite in HIV+ samples has not been examined.

Methods: Potential biomarker correlates of stress were assessed among 100 poor African American and Latina mothers living with HIV (MLH) and demographic-matched HIV- control mothers (n=50). Participants had been enrolled in a randomized controlled trial about 18 months earlier and MLH had either received (MLH-I) or were awaiting (MLH-W) the psychosocial intervention (n=50 each). Cortisol and catecholamines were assayed from home-collected urine and basic health indicators (blood pressure, height and weight, waist-to-hip ratio) were measured during an interview. Psychological symptoms (e.g., depression and posttraumatic stress disorder), general health behaviors and HIV-specific health variables that might serve as potential mediators or moderators of biomarker outcomes were also assessed in a single interview.

Results: Participation was high, biomarkers were correctly collected for 93% of cases, and a complete composite biomarker index (CBI) calculated for 133 mothers (mean age = 42). MLH had a significantly higher CBI than controls, but there was no CBI difference across ethnicity or intervention group. A higher composite was predicted by lower medication adherence among MLH and the composite independently predicted most recent CD4 counts after controlling for known predictor variables (age, years since diagnosis, prior baseline CD4 counts, medication adherence, and depression symptoms). Self-reported health behaviors [sleep, diet, exercise, caffeine and tobacco use, or substance (alcohol and drug) use] were different in controls compared to MLH mothers, nor were these significantly correlated with the biomarker composite.

Conclusions: The study demonstrates acceptability, feasibility and potential utility of community-based biomarker collections in evaluating individual differences in psychosocial stress.

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27.

Extreme (High or Low) Peripheral Urinary Cortisol Associated with Lower Left Hippocampal Volume in Chronically Stressed Mothers of Seriously Ill Children

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Background: Glucocorticoids in the rat brain (cortisol in humans) are released in response to acute and chronic stress. Preclinical work indicates prolonged high levels of glucocorticoids reduces the size of the hippocampus, and meta-analyses support evidence for reduced hippocampal volume in humans with posttraumatic stress disorder (PTSD). However, many (but not all) clinical studies also show *lower* resting cortisol levels in PTSD samples relative to normal controls or samples with Major Depressive Disorder (MDD). The absence of consistent evidence for elevations in cortisol among PTSD patients specifically, and chronically stressed groups more generally, challenges prevailing concepts about the role of stress-induced cortisol release in hippocampal volume.

Methods: Structural magnetic resonance imaging (MRI) exams and neuroendocrine biomarkers (cortisol and catecholamines assayed from a 12-hour overnight urine collection) were assessed in healthy women (N=30) with histories of stress precipitated by their child's diagnosis of a life-threatening illness. A structured clinical interview (SCID) assessed for past and current PTSD and MDD. Cortisol and catecholamines were assayed from.

Results: Linear regression predicting left hippocampal volume (normalized for total brain volume) was significant [$F(1,28) = 4.96, p=.008$]; after controlling for the negative association with mother's age (increased age linked to reduced volume; $p=.02$) and positive association with time since stressor onset (greater months since child's diagnosis of life-threatening illness linked to increased volume; $p=.001$), left hippocampal volume showed a significant quadratic relationship to urinary cortisol (very low and very high cortisol levels were both linked to reduced left volume; $p=.03$). The inverted U-shaped cortisol effect was not dependent upon the presence or severity of PTSD or MDD symptoms, but disappeared if time since stressor onset was removed from the model.

Conclusions: Data indicate hippocampal volume, cortisol levels, and their associations change over time after exposure to a strong stressor and support Sapolsky's (2000) proposal that both pathological hypersecretion and hyposcretion can be damaging to the hippocampus. Future prospective longitudinal studies will be needed to examine the temporal course of these changes to elucidate specific mechanisms.

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28.

Autonomic Nervous System Function Compared in Women with Irritable Bowel Syndrome, Healthy Controls and Major Depressive Disorder

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Irritable Bowel Syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain or discomfort combined with alteration in bowel function. Altered brain-gut interactions and dysfunction of the autonomic nervous system (ANS) contribute to the symptoms seen in IBS. In this study, the primary focus was to look at ANS function in patients with IBS. Our aim was to examine differences in ANS function under resting conditions, comparing the responses of subjects diagnosed with IBS to subjects diagnosed with Major Depression and healthy controls. Measures of sympathetic (SNS) and parasympathetic (PNS) were obtained. Based on previous findings on ANS activity in IBS patients, depressed patients, and IBS patients with comorbid anxiety/depression disorders, we hypothesized that (1) IBS patients will have increased SNS and decreased PNS activity compared to the healthy controls and (2) ANS measures would be similar in the two patient groups.

This study investigated autonomic nervous system function in three subject samples: women diagnosed with Irritable Bowel Syndrome (IBS) (N = 16), Unipolar Major Depressive Disorder (N = 17), and healthy controls (N = 17). Physiological measures of autonomic function including heart rate, systolic blood pressure, and heart rate variability were obtained under resting conditions. Results showed significant main effects (p<0.05) for all the measures. In general, the two patient groups did not differ from each other but showed higher levels of sympathetic and lower levels of parasympathetic nervous system activity compared to the healthy subjects.

The findings (Table 1) have implications for the further understanding of the pathophysiology of patients with IBS, and the similarities in autonomic function between IBS and depressed patients merit further investigation.

Table 1: Means (SE) of Physiological Measures

| Variables | Healthy | IBS | Depressed | P |
|-------------------|----------------------------|----------------------------|----------------------------|----------|
| HR (bpm) | 65.3 (2.30) ^a | 75.2 (2.38) ^b | 71.2 (2.30) ^a | 0.016 |
| SBP (mmHg) | 108.1 (4.58) ^a | 125.0 (4.72) ^b | 118.3 (4.58) ^a | 0.043 |
| LNTP23 | 0.800 (0.038) ^a | 1.106 (0.040) ^b | 1.102 (0.038) ^b | <0.001 |
| LNTP2T | 0.299 (0.008) ^a | 0.342 (0.008) ^b | 0.339 (0.008) ^b | <0.001 |
| LNTP3T | 0.376 (0.006) ^a | 0.314 (0.007) ^b | 0.310 (0.006) ^b | <0.001 |

Note: Groups with different superscripts were significantly different (p < 0.05).

LNTP23 = ratio of low to high frequency HRV; LNTP2T = ratio of low frequency to total power, LNTP3T = ratio of high frequency HRV to total power.

29.

Myeloencephalitis and Silent Thyroiditis: A Case Report

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Background: To define the clinical course of acute myeloencephalitis with coexisting silent thyroiditis in a patient. We review the clinical manifestations, diagnostic evaluation and treatment approaches for this rare case.

Discussion: A 59-year-old woman presented with pneumonia, acute myeloencephalitis, silent thyroiditis, urinary retention, ileus and hyponatremia. Initially, there was decreased muscle strength in upper and lower extremities. Progressive weakness and numbness led to quadriplegia during the first week of her illness. The thyroid gland was enlarged and nontender. Sensory and deep tendon reflexes were diminished in the lower extremities. Babinski sign was positive. Laboratory findings revealed elevated levels of serum IgA and IgM, hyponatremia, hyperthyroxinemia and low radioiodine uptake of the thyroid gland. Imaging studies of the central nervous system and thoracic spinal cord were suggestive of myeloencephalitis. Using prednisone, antibiotics and supportive care, she made a full recovery four months after the onset of her illness.

Conclusion: We hypothesize that she had an autoimmune disorder that transiently caused myeloencephalitis and thyroiditis. Constellation of these findings has not been previously reported.

30.

Pain Catastrophizing Is Associated with an Emotional Stroop Effect

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It has been demonstrated that persistent negative thinking and poor coping impact health. Since cognitive inhibition is normally necessary to control thoughts and actions, inhibitory deficits may underlie rumination or negative interpretation of events, which in turn contribute negatively to health. Specifically, catastrophizing about health-related information may contribute to the experience of distress in chronic physical disorders. The focus of this study was to determine whether impaired cognitive inhibition is linked to pain catastrophizing. We hypothesized that high catastrophizers would have difficulty inhibiting interfering emotional information, which would affect behavioral performance. In a variant of the widely-used Stroop task, 48 undergraduate students were given two lists of words printed in colored ink and were asked to name the color of each word as quickly as possible. This task measures inhibitory control by requiring the subject to suppress the dominant response of reading the word. The lists contained words that were either emotional (e.g., death) or neutral (e.g., salad). Participants' scores on the Pain Catastrophizing Scale (Sullivan, Bishop & Pivik, 1995) were also obtained. This scale measures self-reported rumination about pain, magnification of the experience of pain, and feelings of helplessness about pain, and has been associated with the experience of distress in chronic functional disorders. A comparison of the neutral and emotional conditions revealed an emotional Stroop effect (longer color naming times for emotional relative to neutral words) for high ($p=.017$) but not low catastrophizers. These data associate maladaptive coping

style with impaired cognitive inhibition for emotional material. We suggest that basic cognitive control problems may factor into the experience of distress in clinical populations in which persistent intrusive thoughts are a problem. Inhibitory control of emotionally-charged material may be particularly difficult for these individuals. This poor inhibitory control may lead to greater attention to pain and surrounding emotions, which may in turn affect the experience of pain. The current study is part of a larger effort to examine relationships between cognitive control, emotion, and health outcomes.

31.

Alpha-2 Adrenergic Receptor (α 2AR) Antagonist Modulates EEG Theta-Band and Gamma-Band in Healthy Individuals

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Introduction: Previous research demonstrated that EEG gamma (γ) band response (30-70 Hz) is linked with voluntary shifts of attention, but not automatic or involuntary capture of attention, while theta (θ) band (4-7 Hz) response can be modulated by maintenance of selective attention. Locus coeruleus (LC) activity has often been linked with attention-based processes, such as providing an alert signal to attend to relevant stimuli. Attentional processes have also been linked with event-locked oscillatory brain activity of γ and θ -bands. α -2 adrenergic receptor antagonists (α 2AR) have been found to modulate LC activity. As such, we sought to assess whether administration of yohimbine (Yoh), an α 2AR, would affect γ and θ -band responses to an oddball task, which requires selective attentional processes.

Methods: In a placebo-controlled study, 9 healthy participants (5 female) received Placebo or Yoh prior to completing an auditory oddball task. After being fitted with an EEG cap, low- and high-pitched tones were presented to both of the participant's ears. Participants provided a button press when target tones, which were presented infrequently, occurred in a designated ear. EEG responses were time-locked to the onset of the infrequent target, and averaged across events. Event related spectral perturbation (ERSP) was analyzed using EEGLab.

Results: With Placebo, participants exhibited γ -band activity 100-200ms, and 300-400 ms after onset of the auditory oddball, as well as θ -band activity at 300-400 ms. γ and θ -band activity was significantly greater following administration of Yoh than placebo.

Discussion: Synchronized oscillatory brain activity in θ and γ -bands help coordinate neural signaling, and such coordination is linked to both attention and memory-based processes. Yoh may affect oscillatory brain activity by facilitating norepinephrine (NE) release in LC, which is thought to optimize attention-based behavioral performance. Our data suggest Yoh modulates attentional processes at the neural level. As such Yoh may help further coordinate the neural systems responsible for focusing selective attention in healthy individuals. Additional data (presented at the current conference by Vianna et al.) suggests Yoh may differentially affect such systems in patients with irritable bowel syndrome.

32.

Alpha-2 Adrenergic Receptor Antagonist Modulates EEG Theta-band in IBS Patients

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Introduction: Patients with irritable bowel syndrome (IBS) are hypervigilant to symptom-relevant stimuli and environmental contexts. The locus-coeruleus (LC) plays an important role in attentional processing and performance on attention-based tasks. Low frequency theta (θ) band (4-7 Hz) EEG oscillation is linked to attention and working memory processes. We sought to determine whether untreated patients with IBS have an altered θ band response to an auditory oddball task. We also sought to determine whether IBS patients who were treated with yohimbine (Yoh), an α -2 adrenergic receptor antagonist that modulates LC activity, would demonstrate an altered θ -band oscillatory brain response to the same oddball task.

Methods: In a double-blind cross-over study, ROME II positive IBS patients (N=9) and healthy controls (N=9) received placebo or Yoh (40mg) ~45-min prior to performing an auditory oddball task. EEG was recorded while low- and high-pitched tones were presented to both of the participant's ears. Participants were instructed to provide a button press when target tones, which were presented infrequently, occurred in a designated ear. EEG responses were averaged across oddball events, which were time-locked to the onset of the infrequent auditory target. Event related spectral perturbation (ERSP) was analyzed using EEGLab.

Results: Compared to controls, IBS patients who received placebo had greater response amplitude in the θ -band 300-400 ms after presentation of the stimulus. When IBS patients received Yoh, there was a decrease in θ -band amplitude 300-400 ms after the onset of the TARGET. Conversely, healthy controls exhibited an increase in θ -band activity with Yoh (presented at current conference by Jarcho et al.).

Conclusions: Oscillatory brain activity in θ band is higher in untreated IBS patients than controls, but activity in the θ -band is decreased in patients after administration of Yoh. It is possible that Yoh affects oscillatory brain activity by facilitating norepinephrine (NE) release in LC. It has been proposed that LC-NE helps optimize attention-based behavioral performance. LC and behavioral performance follows an inverted U shape relationship. Our data suggests that the attentional systems at the neural level in untreated IBS patients may be hyperresponsive, and Yoh administration modulates integrated brain oscillations in an inverted U fashion.

33.

Evidence for Increased Responsiveness of an Emotional Arousal Network During Pelvic Visceral Discomfort in Female IBS Patients

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Background: Clinical and neuroimaging evidence suggests an important role of central pain

amplification mechanisms in the pathophysiology of functional pain disorders, including irritable bowel syndrome (IBS). However, the brain regions and circuits underlying this amplification mechanism are poorly understood. This study tested for a functional brain network associated with experimentally-induced pelvic visceral discomfort and characterized differences in the activation of this network between female IBS patients and healthy controls (Ctrls).

Methods: Brain responses to controlled rectal distension were assessed by functional magnetic resonance imaging (1.5 T) in 13 healthy control women and 13 women with IBS-costipation during 5mmHg (Sham), 25 mmHg (Moderate), and 45 mm Hg (High) rectal balloon inflation. Multivariate partial least squares (PLS) was employed to identify distributed patterns of regions that relate to the brain's response to aversive visceral stimulus in IBS patients and Ctrls (task PLS). Path analysis within a structural equation modeling framework was applied to characterize and test for differences in the effective connectivity of an emotional arousal network.

Results: Task PLS identified a distension-related network (73% of total variance, pSham) of homeostatic afferent regions (including the thalamus, bilateral anterior insula (aINS), dorsal mid and anterior cingulate regions), as well as frontal (BA 6) and bilateral parietal (BA 40) cortices. The network also comprised reliable deactivations (High <Moderate<Sham) of limbic and paralimbic regions (including the right amygdala, parahippocampal gyrus, hippocampus, ventral (BA 32/25) and posterior cingulate regions) of visceral and somatic sensory regions (right thalamus, dorsal INS and somatosensory cortex (BA 3/2/4) as well as of bilateral orbital and medial prefrontal (BA 10, 11, 9/32). Effective connectivity analysis of an emotional arousal circuit (Amyg to infragenu cingulate to supragenu cingulate to amygdala) demonstrated a) a reduced feedback inhibition in IBS (Amyg to iACC; -0.88, 0.49, $X^2\Delta=22.3$); and reduced prefrontal inhibition (omPFC to Amyg; -0.51, 0.19, $X^2\Delta=15.7$).

Discussion: When compared with healthy women, female IBS patients have: (1) greater responsiveness of homeostatic afferent brain regions to the actual visceral stimulus, (2) greater engagement of an emotional arousal network and (3) less effective inhibitory modulation of the arousal circuit. Increased responsiveness of an emotional arousal circuit is likely to play an important role in central pain amplification in functional pain disorders, and may explain the common comorbidity of these syndromes with anxiety disorders.

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34.

Brain Networks Underlying Perceptual Habituation to Repeated Application of Visceral Stimuli

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Background: Symptom-related fears and associated hypervigilance toward viscerosomatic stimuli or contexts may play a crucial role in central pain amplification in functional pain disorders, including irritable bowel syndrome (IBS) pathophysiology. Such hypervigilance may be the result of altered attentional and affective modulation of perception (Naliboff et al. 2006) We hypothesized that changes in the activity of specific functional brain networks related to attention and emotional arousal may contribute to the perceptual habituation observed during repeated sessions of stimulation.

Methods: Regional cerebral blood flow (rCBF) was assessed in 12 IBS patients (8women) receiving repeated barostat-controlled rectal distension assessments 6 times over a 12-month period. During the first and last session, PET($H_2^{15}O$) images were obtained during 2 trials each of resting baseline(BL), aversive rectal distension (INF), and expectation of an aversive distension (EXP). After each run, patients rated their visceral sensation on a 20-cm VDVAS of stimulus intensity for each condition (BL, INF, EXP). A behavioral partial least squares analysis was applied to determine whether there was a network of regions correlated the pain intensity ratings of the balloon stimulus during BL, 40 mm, 60 mm and EXP Day 1 and Day 2.

Results: In general, stimulus intensity ratings decreased from Day 1 to Day 2 across all conditions, however, statistically significant decreases were only observed during the inflation condition (mean difference=1.8, $t(11)=3.2$, $p<.01$). Permutation testing indicated two significant “stimulus intensity rating” networks. The first was a network of brain regions associated with the stimulus ratings only during non-INF conditions (BL and EXP) but not INF (34.43%, $p=.008$). This non-INF network included the following regions which were positively correlated with stimulus ratings: anterior insula (aINS), cingulate cortex subregions (dorsal, rostral), prefrontal cortex (PFC) subregions (dorsal, medial and lateral) (BA 9, 10), hypothalamus, parietal, and middle temporal gyrus regions. Regions that were negatively correlated with stimulus intensity included: ventral cingulate regions and posterior cingulate regions (subgenual ACC, posterior cingulate), prefrontal regions (omPFC ;BA 11; dIPFC, BA 46.9), Amygdala, hippocampus, midbrain, periaqueductal gray (PAG), , inferior parietal (BA 40), temporal gyrus and precuneus regions. The second network comprised brain regions correlated with the stimulus ratings only during INF (29.52%, $p=.03$). This INF network comprised brain regions positively with the stimulus ratings included posterior INS, dorsal ACC and posterior cingulate subregions, prefrontal regions (mPFC, BA 9; IPFC, BA 47,46), amygdala, PAG, temporal, pre- and post central gyrus (BA 6/2/,40) and precuneus. Regions comprising this network which were negatively correlated with intensity ratings during INF included hippocampus, cerebellum, omPFC, posterior cingulate, parietal, temporal (BA 21) and pre- and post central gyrus (BA 4/ 3) regions.

Discussion: Distinct, yet overlapping networks were engaged during non-INF and during INF conditions. Even though the two networks involved similar general regions of INS and PFC, the networks differed in subregions within these general brain areas. These findings suggest that the perceptual habituation to the visceral stimulus is related to specific changes within these two networks over time.

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35.

Functional Network Activation During Pelvic Visceral Distension: A Meta-Analysis

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Aims of the Investigation: Irritable bowel syndrome is one of the most common chronic functional pain syndromes, characterized by chronic abdominal pain/discomfort associated with alterations in bowel habits. IBS is frequently associated with other chronic pain syndromes such as fibromyalgia, interstitial cystitis and migraines. Recent neuroimaging research suggests that

altered central nervous system responsiveness and associated alterations in pain modulation, emotional arousal and attentional circuits may play an important role in symptom generation. However, reaching a consensus about published data has proved difficult due to the variety of experimental paradigms, analytic techniques, and reporting practices. The aim of this study was to apply a quantitative meta-analysis to map the consistent findings for supraliminal pelvic visceral distension (INF) in IBS and healthy controls (Ctrls).

Methods: Published studies were included only if significant foci for within group analyses of INF for IBS or Ctrls were reported. Results for IBS and Ctrls were pooled and compared using voxel-based activation likelihood estimation (ALE) meta-analysis. Significance was determined using permutation testing and thresholding results at a false discovery rate of 1%.

Results: Twelve published studies met inclusion criteria and yielded tabulated coordinates for 10 INF contrasts for IBS patients and 7 INF contrasts for Ctrls. The ALE analysis of IBS patients incorporated 148 foci and the healthy control analysis used 96 foci. The number of clusters and their total volume that resulted from the respective IBS and Ctrls ALE analyses reflected the greater number of studies contributing to the IBS analysis (IBS clusters = 10, Ctrls clusters = 6, total volume IBS = 13,552 mm³, and total volume Ctrls = 17,600 mm³). During INF, IBS patients and Ctrls showed consistent activity in rostral (BA 32) and dorsal (BA 24) anterior cingulate cortices. Bilateral activity in amygdala, midbrain, hypothalamus, and thalamus was associated with IBS patients during INF but not Ctrls. Substantial overlap between IBS and Ctrls was observed for bilateral anterior insula with IBS generally showing greater spatial extent with the exception of one cluster in the left anterior insula that was greater in Ctrls. For IBS patients, activity in the right dorsal PFC (BA 10) and superior frontal gyrus (BA 6) was related to INF where as activity in inferior parietal regions (BA 40) was associated with INF in Ctrls.

Conclusions: These preliminary results suggest greater activation of limbic arousal circuits (amygdala, dorsal pons) and differences in homeostatic afferent circuits (insula, thalamus) in IBS patients.

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36.

Does Placebo-Induced Analgesia Result in a Change in Sympathetic Nervous System Reactivity?

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Background: Although placebo effects have been documented in numerous clinical and experimental studies, the great majority of those studies focus solely on self-reported symptom improvement. With the exception of recent neuroimaging studies, few experiments have investigated whether self-reported placebo responses are accompanied by corresponding physiological changes. Changes in sympathetic nervous system (SNS) activity, including galvanic skin conductance response, have been described as an index of the experience of pain. It should therefore be possible to measure placebo-induced analgesia both objectively, through a decrease in skin conductance response, and subjectively, through self-reported reduction in pain. The current research sought to investigate this relationship.

Methods: Healthy female participants (n=27) received a 12-min continuous thermal stimulus and rated their pain at 25sec intervals. Unbeknownst to participants, a computerized algorithm adjusted the temperature at each interval to produce and maintain participant-specific moderate

levels of pain. A topical placebo analgesic was applied, and the same stimulus profile was repeated, while participants again rated their pain. Number of skin conductance responses that occurred during each thermal stimulus was then assessed.

Results: The computerized algorithm successfully maintained a participant-specific moderate level of pain during the initial thermal stimulus. Participants reported significantly less pain (subject measure of analgesia) and, and fewer skin conductance responses (objective measure of analgesia) when the thermal stimulus was coupled with the placebo analgesic than when it was not.

Conclusions: Results indicate that placebo-induced analgesia can be measured objectively with SNS reactivity, as well as subjectively with self-report, providing further support that placebo analgesia occurs both in the mind and the body.

37.

Sensorimotor Gating Deficit in Children Exposed to Trauma

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Objectives: In PTSD, symptom cluster D suggests deficient capacity to inhibit excessive cognitive, perceptual, affective and motor responses. This may reflect deficiency of *sensorimotor gating*, the capacity of nervous systems to protect stimulus processing from interference by motor excitation. Sensorimotor gating, measured as prepulse inhibition (PPI) of startle, is deficient in veterans exposed to combat who develop PTSD, but not in civilians exposed to single traumas. This study evaluates PPI in traumatized children in a way that can be contrasted with studies of adults.

Methods: We compare PPI of startle in 25 children, 17 with DSM-IV criteria for PTSD, who had experienced a single traumatic event and 16 children, matched for age, without trauma history.

Results: PPI of startle is reduced in traumatized children; those who meet diagnostic criteria for PTSD have the greatest deficit. Hence, there is a significant linear contrast when children with PTSD (the least PPI), traumatized children without PTSD (greater PPI), and non-traumatized children (the greatest PPI) are compared ($p = 0.024$).

Conclusions: Trauma per se induces disruption of sensorimotor gating; if PTSD develops, then this disruption is more severe. Children appear to be more vulnerable to the effects of trauma in that a single episode may induce lasting change; in contrast, in post-adolescent individuals, chronic trauma is necessary to produce the same effects.

38.

Mediation of Pain by Applied Mindfulness Training: An fMRI Study

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Background: Meditation has been shown to have positive effects on health, with studies on the effects of meditation on patient groups suggesting a general antinociceptive effect. Mindfulness forms of meditation have been shown to reduce self-reported pain in chronic pain patients, and a reported case study showed functional magnetic resonance imaging (fMRI) data supporting an advanced meditator's claim that he feels no pain during meditation. However, the effect of meditation on pain responses in healthy subjects hasn't been well characterized, particularly in novice meditators.

Methods: We are assessing the effect of mindfulness meditation training on responses to acute thermal pain in healthy women as measured by subjective report and fMRI. 24 right-handed female subjects, with little or no previous meditation experience, are being assessed for acute thermal pain thresholds and trait mindfulness, then are randomly assigned to standardized 8-week mindfulness training or wait-list control groups. fMRI assessment of responses to acute thermal pain follow the training and wait-list periods.

Pilot and Preliminary Study Results: Prior to beginning the study, 10 pilot subjects (4 experienced meditators, 6 non-meditators) were run through an abbreviated version of the study screening and fMRI protocol. Pilot fMRI data analyses (SPM5) showed applied mindfulness training compared to a "non-mindful" state is consistent with increased rostral and infragenu anterior cingulate cortex activity indicating relatively greater cognitive/affective resources employed in dealing with the painful stimuli. The applied mindfulness condition was also consistent with decreased activity in the anterior insula contralateral to the applied thermal stimuli, indicating a reduction in the sensory/affective impact of the painful stimuli. Analyses of the interim data from the completed phases of the ongoing study showed a pattern of results similar to those of the pilot study.

Conclusions: Results indicate applied mindfulness training increases the centrally-mediated cognitive/affective responses to acute pain, with limited impact on the sensory response. Ongoing data analyses are supporting and extending the current findings showing neural correlates of pain-mediating properties of applied mindfulness training.

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39.

A Sex-Dependent Brain Network Related to Cardiovagal Activity in Irritable Bowel Syndrome

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Objectives: To characterize sex differences in the brain networks related to cardiovagal activity during rectal balloon distension in patients with irritable bowel syndrome (IBS).

Methods: 36 IBS patients (Rome I positive, 18 females) received H₂¹⁵O-PET scans during a resting baseline, aversive rectal balloon distension, and expectation of distension conditions. Two lead ECG was continuously recorded during the three conditions. Peak power high frequency (ppHF), the vagal component of heart rate variability, was determined using Fast Fourier Transformation. Mean ppHF values were log transformed and a repeated measures analysis within the general linear model framework was applied to assess sex differences in ppHF across conditions. Multivariate partial least squares (behavioral PLS) was used to identify distributed patterns of brain activity that were functionally connected with ppHF across sex and conditions.

Results: Female patients had significantly higher mean ppHF across conditions, $p < 0.01$. Behavioral PLS revealed a significant sexually dimorphic network, $p < 0.05$. Regions in the network correlating more positively with ppHF in females compared to males included: hypothalamus, orbitofrontal cortex (BA 11), periaqueductal gray, and posterior insula. Regions correlating negatively with ppHF in females compared to males included the rostral anterior cingulate cortex, dorsolateral prefrontal cortex (BA 46), and dorsal prefrontal cortex (BA 10).

Conclusions: A sex dependent central network associated with cardiovagal responses to a pelvic visceral stimulus can be identified in IBS patients. This network involves regions of the emotional motor system (PAG, hypothalamus), interoceptive (posterior insula) and as well as prefrontal regions. The sex-specific network may explain previously reported differences in ANS responses between males and females. Sex related differences in ANS function may provide targets for future sex-specific therapies for this common chronic disorder.

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40.

Early-Life Stress Is Associated with a Neural Bias Towards Emotionality

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Adverse rearing environments can produce long-lasting change in responsivity to arousing stimuli and the ability to regulate emotion. Children who had been institutionally reared during infancy ($n=19$) were scanned using fMRI while engaged in an emotional go-nogo task (Hare et al., 2005, 2008), which measures cognitive control in the context of highly arousing stimuli. There were no group differences in accuracy on the task ($F(1,35) = 0.45$) relative to an age-matched sample ($N=18$). However, there were differences in the neural systems that the two groups recruited to perform the task. Unlike typical rearing, which resulted in a recruitment of a

perceptuo-cognitive network, including fusiform gyrus and inferior frontal gyrus, early life adversity was associated with a greater tendency to engage an emotional network, including amygdala and ventral medial prefrontal cortex. Thus, although behavioral performance did not differ between groups, the neural circuit recruited by the previously institutionalized children tended towards an emotional one. These findings extend our earlier work showing an association between early life stress and atypically large amygdala volume and suggest that stressful experiences in infancy increase the bias towards recruitment of emotional systems.

41.

COMT 4680 Gene Polymorphism Is Associated with Altered Acoustic Startle Response Modulation in Patients with Irritable Bowel Syndrome (IBS) and Fibromyalgia (FM)

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Background: Modulation of the acoustic startle response (ASR) by threats (fear potentiation [FP]) and prepulse inhibition [PPI] are objective, non-invasive measures of amygdala complex responses to specific contexts or stimuli. These responses are enhanced in IBS (Naliboff Psychosom Med 2008). Acoustic startle modulation is in part related to dopaminergic mechanisms (DA) in the prefrontal cortex (PFC), where synaptic DA levels are closely related to catechol O-methyltransferase (COMT) levels, which in turn are influenced by the Val158Met polymorphism (COMT 4680). The Val/Val allele is associated with high activity of COMT, low synaptic DA, and high presynaptic phasic DA release. Met carriers [Val/Met, Met/Met] show high synaptic DA and reduced phasic DA release. The Met allele has been associated with reduced central opioid release and greater vulnerability for chronic pain disorders, including migraine and FM.

Aims: To compare the effect of COMT 4680 on PPI and on FP of the ASR in IBS and FM patients vs. healthy controls (Ctrls).

Methods: Fifty-six female subjects (18 IBS, 10 IBS+FM, 6 FM, and 23 Ctrls) underwent ASR and genetic salivary testing for COMT 4680 status. SNP and polymorphism genotyping was performed using allelic discrimination assay and gene mapper software respectively. ASRs were measured from EMG activity of the orbicularis oculi muscle following a 104dB, 50 ms burst of white noise. **PPI** was measured from startle stimuli which were preceded by a short, innocuous tone (25ms, 1000Hz, 75dB SPL, 4ms rise and fall times). **FP** was measured during 3 cued threat conditions: safe (no stimulation), anticipation (possible stimulation), and imminent threat of an electrical abdominal (IBS) or bicep (FM±IBS) shock.

Results: Individuals were grouped by diagnosis (IBS±FM vs. Ctrls) and COMT 4680 status (Val/Val vs. Met carriers). In both groups, both PPI ($p<0.001$) and FP ($p=0.03$) was significantly higher in Met carriers compared to Val/Val subjects. A significant 3-way interaction was seen between Met status, Diagnosis, and Threat Condition. In patients, Met carriers showed enhanced FP during imminent threat ($p<0.001$).

Conclusion: Reduced presynaptic phasic DA release in Met carriers may result in impaired ability of the PFC to downregulate startle responses (and amygdala complex activity) during

threat and increased attention to a prepulse. These altered early response mechanisms to potential and symptom related threats may play a role in central pain amplification and hypervigilance, and may partially explain the reported greater prevalence of the Met allele in chronic pain syndromes.

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42.

Associations Between Maternal and Child Responses to Laboratory Tasks Inducing Visceral and Somatic Pain

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The water load task (WLT) has been shown to induce clinically relevant symptoms in children and adults with functional abdominal pain. Previous research indicates that mother's responses to their children during the WLT significantly impacts laboratory-induced symptoms in children. However, no prior work has examined the association between mother's responses to the WLT and their children's responses to this visceral pain task. This investigation tested mother-child laboratory pain relationships in 13 children (10 girls) with recurrent abdominal pain (RAP) and 12 healthy children (6 girls) aged 12-17 years, and their mothers. Children and mothers each completed the WLT separately. In addition, children and mothers separately completed the cold pressor task (CPT), as a measure of somatic pain. As shown in Table 1, there were stronger correlations for anticipated pain, anticipatory anxiety, and pain intensity for the WLT among healthy mother-child pairs versus RAP mother-child pairs. However, there was a stronger relationship for pre-WLT negative mood among the RAP versus the healthy group. Anxiety during the CPT was more strongly correlated for the RAP compared to the healthy group. These findings suggest differential associations for RAP and healthy mother-child pairs for laboratory-induced visceral and somatic pain.

Table 1. Mother-child correlations for the RAP and healthy groups

| | RAP | Healthy |
|--------------------------|------|------------------|
| Anticipated pain WLT | -.27 | .65* |
| Anticipatory anxiety WLT | .38 | .60 [†] |
| Pain intensity WLT | -.18 | .61 [†] |
| Negative Mood WLT | .65* | -.34 |
| Anxiety CPT | .50* | -.16 |

* $p < .05$; [†] marginally significant

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