SECOND ANNUAL NEUROSCIENCE, BEHAVIOR AND HEALTH FORUM

The University of Vermont
College of Medicine

Davis Center Grand Maple Ballroom

February 6-7, 2012

Presented by the Vermont Chapter of the Society for Neuroscience and the Neuroscience, Behavior and Health Spire

February 6, 2012

Dear Colleagues,

Welcome to the Second Annual Neuroscience, Behavior and Health Forum. Presented by the Vermont Chapter of the Society for Neuroscience, and in cooperation with the NBH Steering Committee, this meeting has evolved from the annual "Neuroscience Forum" meetings held at the University of Vermont since 2005. By expanding the scope of this annual meeting we hope to achieve multiple goals:

- To help implement the goals of the Neuroscience, Behavior and Health Spire at UVM by providing a forum to allow researchers from the diverse areas encompassed by the NBH Spire to learn about each other's work. A particular focus is on facilitating communication of graduate students with faculty and other graduate students they would otherwise be unlikely to meet.
- To enhance the ability of participants to earn extramural funding in this especially challenging funding environment by fostering new and creative collaborations.
- To provide a sense of cohesion and common purpose amongst the diverse community of researchers comprising the NBH Spire at UVM.
- To engage the greater community by inviting the participation of high school students from throughout Vermont. This year we have eight students who will participate in guided activities tailored to foster their early career development.
- To engage the greater community by inviting participation of researchers from regional Colleges and Universities.

Thank you for attending!

Sincerely,

Tony Morielli, Ph.D.
President, Vermont Chapter of the Society for Neuroscience
Associate Professor
Department of Pharmacology
The University of Vermont College of Medicine

SECOND ANNUAL
NEUROSCIENCE, BEHAVIOR AND HEALTH FORUM, 2012

MONDAY, FEBRUARY 6

3:00	Poster setup
4:25	Introduction
4:30	**Keynote presentation:** Michael D. Rugg, PhD University of Texas at Dallas Distinguished Chair in Behavioral and Brain Sciences Co-Director, Center for Vital Longevity: *The Cognitive Neuroscience of Human Memory: A Perspective from fMRI*
5:30	Reception with pay-bar and *Hors d'oeuvres*

TUESDAY, FEBRARY 7

8:45 – 9:45	Posters
Session I: Chaired by	***Geoffrey Schaubhut and Michelle McNamara***
9:45	Hugh Garavan: *The Neurobiology of Cognitive Control in Addiction, Addiction Risk, and Treatment Outcome*
10:00	*Greg Lieberman*: *Diffusion Tensor Imaging for the study of Chronic Pain*
10:15	Jason Fuchs: *Role of Kv 1.2 eyeblink classical conditioning*
10:30	Allan Gulledge: *Selective serotonergic excitation of callosal-projection neurons*
10:45	Coffee and snacks
Session II: Chaired by	***Nathan Jebbett and Simone Otto***
11:00	Matthew Rand: *Neurotoxicity of Methylmercury: a fundamental activity for Cytochrome p450 enzymes in neuroprotection during development*
11:15	Anthony Pappas: *Kv1 and Kv2 channel currents are differentially suppressed in parenchymal arteriolar myocytes obtained from subarachnoid hemorrhage model animals*
11:30	Nabanita Mukherjee: *Cyclophosphamide induced taste disruption in mice*
11:45	Jose Maldonado: *Latest Developments from MBF Bioscience for Neuroscience Research*
12:00-1:00	**Lunch (sandwich fixin's and salad buffets) and posters**
1:00-2:00	Posters
Session III: Chaired by	***Gregory Lieberman and Vanessa Ochoa***
2:00	Julie Dumas: I*ncreased frontal activation during working memory performance in postmenopausal women with cognitive complaints*
2:15	Simone Otto: *PSCA interaction with α7 nicotinic acetylcholine receptors in the avian ciliary ganglion*
2:30	Abbie Chapman: *Mechanisms of the Cerebral Myogenic Vasodilatory Response to Acute Hypotension in Female Rats*
2:45	Coffee and snacks
Session IV: Chaired by	***Michael Williams and Abbie Chapman***
3:00	Malou Schreurs: *The maternal blood-brain barrier permeability in preeclampsia: involvement of lectin-like oxidized low-density lipoprotein receptor-1 and peroxynitrite generation*
3:15	Meghan Eddy: *The effects of voluntary exercise on set-shifting and discrimination in male Wistar rats*
3:30	Laure Case: *Genetics of experimental allergic encephalomyelitis supports the role of T helper cells in multiple sclerosis pathogenesis*
3:45	**Awards and closing remarks**

Second Annual Neuroscience, Behavior and Health Research Forum

February 6 – 7, 2012

Grand Maple Ballroom, Davis Center, University of Vermont

Monday, February 6
4:30 pm

The Cognitive Neuroscience of Human Memory: A Perspective from fMRI

Michael D. Rugg, Ph.D.

University of Texas at Dallas
Distinguished Chair in Behavioral and Brain Sciences

Reception to follow keynote address

Tuesday, February 7
8:30 am – 4:00 pm

Poster Sessions and Talks

For registration please email nbh@uvm.edu

http://www.uvm.edu/~vtsfn/forum2012.php

Sponsored by the Neuroscience Graduate Program, Vermont Chapter of the Society for Neuroscience, and the Neuroscience, Behavior and Health Initiative.

Abstracts

Platform Talks

THE NEUROBIOLOGY OF COGNITIVE CONTROL IN ADDICTION, ADDICTION RISK, AND TREATMENT OUTCOME

Hugh Garavan
Departments of Psychiatry and Psychology, University of Vermont, USA.

This talk will describe neuroimaging research into executive functions, that is, those cognitive processes that guide and control our behavior. Many clinical conditions are characterized by deficits in control and we will focus on addiction with research showing neuroimaging differences between drug users and controls. We will also present evidence of cognitive control deficits that precede drug use suggesting that impaired control may be a risk factor for drug use and subsequent drug abuse. Finally, at the other end of the addiction life cycle, we will show evidence that successful abstinence is characterized by improved control reflected in changes on prefrontal function and grey matter volume.

DIFFUSION TENSOR IMAGING FOR THE STUDY OF CHRONIC PAIN

Gregory Lieberman, Richard Watts, Trevor Andrews, Christopher Filippi, Magdalena Naylor
Department of Psychiatry, Neuroscience Graduate Program

The brains of patients with chronic pain frequently exhibit a variety of differences in both functional activity and the structural organization of gray matter. What is not yet known is whether changes in the characteristics of neural white matter play a role in the etiology of chronic pain. The primary goal of this research is to assess whether there are differences in the characteristics of white matter in the brains of patients with chronic pain. Additionally, we will use Diffusion Tensor Imaging (DTI) to determine whether psychological interventions such as cognitive-behavioral therapy can reverse these differences. This platform talk will focus on the methodology, analysis, and interpretation of DTI in the study of the neural mechanisms behind chronic pain.

THE ROLE OF KV1.2 REGULATION IN EYEBLINK CONDITIONING

Fuchs, JR[1], Williams, MR[2,3], Morielli, AD[2], & Green, JT[1]
Department of Psychology[1], Department of Pharmacology[2], Neuroscience Graduate Program[3]

Converging evidence suggests that Kv1.2 (a voltage-gated potassium ion channel alpha subunit) regulation may support the expression of cerebellar dependent learning. In eyeblink conditioning (EBC), a neutral conditioned stimulus (CS) is paired with an unconditioned stimulus (US). After repeated pairings, the CS elicits a conditioned response (CR) in the form of an eyeblink that occurs before the onset of the US. The cerebellum is the key locus of EBC-related plasticity. An integral part of acquisition and expression of CRs is the disinhibition of one of the deep cerebellar nuclei, the interpositus nucleus (IPN), through removal of Purkinje cell (PC) inhibition. When PC inhibition is lifted, CS projections to the IPN are strengthened allowing the CS to drive CRs. Removal of PC inhibition is usually achieved by depression of PCs through direct CS and US inputs. However, basket cells (BCs), inhibitory interneurons in cerebellar cortex can powerfully influence PC output. Kv1.2 is densely expressed on axon a terminal of BCs. Research has shown that reducing the surface expression of Kv1.2 increases inhibitory current in PC. Blocking Kv1.2 via infusions of tityustoxin-Kα in cerebellar cortex increased the expression of CRs. Secretin acts as a retrograde messenger from PCs to BCs and through intracellular signaling cascades, reduces the surface expression of Kv1.2 at the BC axon terminal surface. Infusions of secretin into cerebellar cortex also facilitated the expression of CRs. Both of these results are consistent with the hypothesis that increasing BC inhibition of PCs disinhibits the IPN and facilitates EBC.

SELECTIVE SEROTONERGIC EXCITATION OF CALLOSAL-PROJECTION NEURONS

A.T. Gulledge and Daniel Avesar
Physiology and Neurobiology, Dartmouth Medical School, Lebanon, NH

Serotonin (5-HT) is a neurotransmitter critical for cognitive function, and disruption of serotonergic signaling in the prefrontal cortex (PFC) is linked to schizophrenia and other psychiatric disorders. We tested the serotonergic responsiveness of cortical pyramidal neurons in the mouse PFC, and found 3 distinct response types: 1A-dependent inhibitory responses (84%), 2A-dependent excitatory responses (9%), and biphasic responses in which excitation followed brief inhibition (5%). Relative to 5-HT-inhibited neurons, those excited by 5-HT had physiological properties characteristic of callosal/commissural (COM) neurons that project to the contralateral cortex. Therefore, we used retrograde fluorescent labeling to identify COM neurons and neurons projecting to the pons (CPn neurons). 5-HT generated excitatory or biphasic responses in all 5-HT-responsive COM neurons (n = 24). Conversely, CPn neurons were universally inhibited by 5-HT (n = 17). Serotonergic excitation of COM neurons was blocked by a 2A antagonist (MDL 11939; n = 5), while serotonergic inhibition of CPn neurons was blocked by a 1A antagonist (WAY 100635; n = 4), confirming a role for these two receptor subtypes in regulating pyramidal neuron activity. Selective serotonergic excitation of COM neurons was not layer-specific, as COM neurons in both layers 5 and 2/3 were selectively excited relative to their non-labeled pyramidal neuron neighbors. Because neocortical 2A receptors are implicated in the etiology of schizophrenia, we propose that COM neurons may represent a novel cellular target for intervention in psychiatric disease.

NEUROTOXICITY OF METHYLMERCURY: A FUNDAMENTAL ACTIVITY FOR CYTOCHROME P450 ENZYMES IN NEUROPROTECTION DURING DEVELOPMENT

Matthew D. Rand, Cecon T. Mahapatra, Amanda Burton.
Department of Anatomy and Neurobiology, College of Medicine, University of Vermont, Burlington, VT. 05405

Abstract:

Methylmercury (MeHg) is a ubiquitous environmental neurotoxin. Historic accidental poisonings in Japan and Iraq have affirmed that the fetal nervous system is the preferred target for MeHg toxicity. Today, a more widespread concern for MeHg exposure comes with consumption of dietary fish. Estimates by the Centers for Disease Control and Prevention (CDC) put more than 300,000 newborns per year potentially affected by harmful prenatal MeHg exposure. A health-related conundrum therefore exists in how to advise the public on consumption of fish. At odds are the potential harmful effects of MeHg and the beneficial effects of nutrients, particularly omega-3 fatty acids. The unclear risks versus benefits of fish in the diet are exacerbated by contrasting results of two large-scale epidemiological studies on prenatal MeHg exposure: the Faroe Islands study and the Seychelles Childhood Developmental Study (SCDS). In the Faroese cohort, significant adverse effects in neurological tests of reflexes, cognition, and auditory-evoked responses were found in children exposed to prenatal mercury. In contrast, no significant correlation between maternal MeHg levels and neurological deficits is found in children of the SCDS. With this stark contrast in results, genetic variation in susceptibility to MeHg in these cohorts has, surprisingly, not been investigated, despite obvious differences in ethnic backgrounds: the Seychellois being of mixed African descent and the Faroese being Caucasian. We have sought to identify fundamental MeHg tolerance genes using the Drosophila model for genetic and molecular dissection of a developmental MeHg tolerance trait. We observe an autosomal dominant MeHg tolerance trait in both wild-derived and laboratory selected MeHg-tolerant strains of flies. Using transcript profiling of larval brains of tolerant and non-tolerant strains of flies we revealed a profile whereby tolerance to MeHg corresponds with an overall greater number of upregulated transcripts. Functional annotation cluster analyses showed enrichment for monooxygenases/oxidoreductases, which include the Phase I metabolism Cytochrome p450 (CYP) enzyme family members. Among the ten CYPs upregulated in tolerant strains, CYP6g1, previously identified as the global DDT resistance allele in flies, was the most highly expressed and responsive to MeHg. We have now characterized induced expression of CYP6g1, and its human homolog CYP3A4, in transgenic flies. We find that elevated expression of either of these CYPs can rescue development of the fly in the presence of MeHg. Remarkably, developmental tolerance is invoked with expression of CYPs limited to the developing nervous system. Furthermore, data from the human HapMap project show that polymorphic variants of human CYP3A4 that predict high and low activity enzymes, respectively, sort to African and Caucasian populations, potentially contributing to the MeHg susceptibility differences of Seychellois and Faroese populations. This study has broad implications for translational studies that resolve a genetic basis for MeHg susceptibility among individuals and assist in the scientific basis of MeHg risk assessment. (Funding: NIEHS R01 ES015550 awarded to M.D.R.)

K_V1 AND K_V2 CHANNEL CURRENTS ARE DIFFERENTIALLY SUPPRESSED IN PARENCHYMAL ARTERIOLAR MYOCYTES OBTAINED FROM SUBARACHNOID HEMORRHAGE MODEL ANIMALS

Anthony C. Pappas, Masayo Koide, Kevin P. O'Connor, Greg J. Smith, George C. Wellman

Deparment of Pharmacology, Neuroscience Graduate Program, University of Vermont College of Medicine, Burlington, VT 05405

Cerebral vasospasm is a common vascular deficit which arises following subarachnoid hemorrhage (SAH). We have previously demonstrated that acute application of the blood component oxyhemoglobin (Oxyhb) causes vasoconstriction of brain surface arteries due to suppression of smooth muscle voltage-dependent K^+ (K_v) channels. Oxyhb-induced K_v channel suppression occurs via a mechanism involving matrix metalloprotease (MMP) activation, shedding of heparin-binding EGF-like growth factor (HB-EGF) and EGF receptor activation. We have also recently observed enhanced constriction of brain cortex parenchymal arterioles (PAs) obtained from SAH model animals. In the present study, we tested the hypothesis that HB-EGF-mediated K_v channel suppression contributes to enhanced PA constriction after SAH. The conventional whole-cell patch clamp technique was used to measure correolide-sensitive K_v1 and stromotoxin-sensitve K_v2 channel currents in PA myocytes isolated from control and SAH model rats. Both K_v1 and K_v2 channel currents were significantly reduced in myocytes from SAH animals, however transcript levels for K_v1 and K_v2 channel subtypes were similar between groups. Treatment of cells with the MMP inhibitor, GM-6001, or CRM197, which disrupts HB-EGF signaling, increased K_v currents in myocytes from SAH, but not control animals. Further, isolated arterioles from SAH animals exhibited greater dilations to GM-6001, or heparin, another inhibitor of HB-EGF signaling, compared to arterioles from control animals. In conclusion, our data demonstrate that K_v1 and K_v2 channel subtypes are functionally expressed in PA myocytes and that these channels are suppressed following SAH.

Supported by the NIH R01 HL078983, P01 HL095488 and the Totman Medical Research

CYCLOPHOSPHAMIDE INDUCED TASTE DISRUPTION IN MICE

Nabanita Mukherjee and Eugene R. Delay
Department of Biology, University of Vermont, Burlington, VT 05405

Clinical studies have reported taste dysfunctions developing in patients undergoing chemotherapy. This adverse side effect is a major concern for the doctors and patients since disrupted taste can reduce appetite, cause malnutrition, delay recovery, and affect quality of life. Cyclophosphamide is a common atenoplastic drug used during chemotherapy and is thought to affect taste through learned tasted aversions. This study asked whether cyclophosphamide also alters umami taste sensory functions and disrupts taste epithelium of mice. Behavioral tests focused on taste acuity, assessed by the ability of mice to discriminate between the taste qualities of two umami substances, monosodium glutamate (MSG) and inosine 5'-monophosphate (IMP), and taste sensitivity, assessed by detection thresholds of MSG and IMP, after an IP injection (75mg/kg) of cyclophosphamide (CYP). The behavioral results revealed a two-phase disturbance in taste acuity and loss of sensitivity, the first phase occurring within 2-4 days after injection and a second occurring 9-12 days after injection. The number of fungiform papillae (with and without pores) decreased immediately after injection and did not begin to recover until 12 days after injection. Circumvallate taste buds began to show disturbances by 8 days after injection and evidence of recovery beginning 12 days after injection. These findings suggest the initial behavioral deficits may be due to cytotoxic effects of the drug on taste sensory tissues whereas the second phase may be due to a disturbance of the taste cell replacement cycle.

INCREASED FRONTAL ACTIVATION DURING WORKING MEMORY PERFORMANCE IN POSTMENOPAUSAL WOMEN WITH COGNITIVE COMPLAINTS

Julie Dumas
Clinical Neuroscience Research Unit, Department of Psychiatry, University of Vermont

Cognitive aging research has begun to focus on cognitive changes in middle age in an effort to identify adults who are at a higher risk of developing cognitive deficits. One group of subjects who are at increased risk for pathological aging are those who report subjective cognitive complaints but perform normally on neuropsychological tests. Prior studies have shown older adults with cognitive complaints have morphological and functional changes relative to older adults with no complaints (Rodda et al. 2010; Saykin et al. 2006). No study has thus far examined the presence and functional consequences of cognitive complaints in a middle aged sample of subjects. Twenty two postmenopausal women aged 50-60 completed a cognitive complaint battery of questionnaires (Saykin et al., 2006). Twelve women were categorized as cognitive complainers because then endorsed more than 20% of the items on the cognitive complaint battery and ten were non-complainers. All subjects then took part in a functional MRI scanning session during which they completed a visual verbal N-back test of working memory. Results showed no differences in working memory performance between complainers and noncomplainers. However, complainers had greater activation ($p < .01$) in the dorsolateral prefrontal cortex (BA 9) as well as in the anterior cingulate cortex (BA 32) relative to the noncomplainers. We interpret this increased activation as compensation in the complainer group such that they recruited additional brain regions to perform the task at the same level as the noncomplainers. This is the first study to show functional activity changes in cognitive complainers in a middle aged group of women.

Support Contributed By: NIA K01 AG030380, NIA R01 AG021476, GCRC M01-00109, DoE SC 0001753

PSCA INTERACTION WITH α7 NICOTINIC ACETYLCHOLINE RECEPTORS IN THE AVIAN CILIARY GANGLION

Simone Otto and Rae Nishi
Neuroscience Graduate Program, University of Vermont

Nicotinic acetylcholine receptors (nAChRs) are involved in signaling throughout the nervous system and α7 subunit containing nAChRs are of particular interest because of their high calcium permeability. Nicotinic signaling may be modulated by the prototoxin family of Ly6-related molecules that share structural homology with snake venom neurotoxins. One prototoxin, Prostate Stem Cell Antigen (PSCA), is upregulated in the ciliary ganglion during development. PSCA over-expression decreases nicotine-induced increases in intracellular free calcium and rescues choroid neurons from death (Hruska et al J Neurosci 2009 47:14847-54). I hypothesize that PSCA acts by binding to α7nAChRs. The ciliary ganglion contains ciliary neurons that develop pseudospines where α7nAChRs cluster and choroid neurons expressing somatostatin-like immunoreactivity (SOM-LIR) whose α7nAChRs are dispersed. By transducing ciliary ganglion neurons with V5-epitope-tagged PSCA in an RCASBP(A) chicken retrovirus, colocalization of PSCA with α7nAChRs was analyzed in 3D images on live-labeled neurons using Delta Vision deconvolution microscopy. PSCA shows two patterns of colocalization and quantification of co-localization using Pearson's index shows a bimodal distribution. Morphological criteria and SOM-LIR, confirmed that a statistically significant stronger colocalization is observed in ciliary vs choroid neurons. Pseudospine loss after culturing neurons one day leads to loss of colocalization and preliminary data from qPCR of cDNA made from cocultures containing neurons and Schwann cells indicates Schwann cells upregulate PSCA. Thus, V5-PSCA colocalizes with α7nAChRs primarily on pseudospines in ciliary neurons, potentially modulating α7nAChR signaling or trafficking.

Funding: DA17784 (RN); 1RC1DA028173 (RN); 5 P20 RR016435 to the UVM Neuroscience COBRE Core Facility

MECHANISMS OF THE CEREBRAL MYOGENIC VASODILATORY RESPONSE TO ACUTE HYPOTENSION IN FEMALE RATS

Abbie Chapman, Siu-Lung Chan and Marilyn Cipolla
Department of Neurology, Ob/Gyn & Repro Sci, University of Vermont

Cerebral blood flow autoregulation (CBFAR) functions to maintain constant blood supply to the brain despite fluctuations in blood pressure (BP). Myogenic vasodilation (MV) to hypotension is a critical component of CBFAR. We investigated the influence of pregnancy on MV and the autoregulatory response to acute hypotension. Autoregulation in response to hemorrhagic hypotension was measured in anesthetized and mechanically ventilated nonpregnant (NP) and late-pregnant (LP, n=8/group) SD rats. Posterior cerebral arteries (PCAs) from NP and LP rats were cannulated in an arteriograph chamber. MV was measured in the absence (n=10/group) or presence of the NOS inhibitor L-NNA (n=7/group) by decreasing pressure from 125 to 5 mmHg and recording luminal diameter. CBF became significant vs. baseline at 90 mmHg in NP animals; BP decreased to 60 mmHg before CBF was different vs. baseline in LP animals, suggesting CBF during hypotension is more stable during pregnancy. PCAs from NP and LP rats developed similar myogenic tone at 125 mmHg. As pressure decreased, LP PCAs dilated, becoming significantly larger at 50 mmHg vs. baseline. This dilation diminished with L-NNA treatment. NP PCAs dilated less and L-NNA did not affect MV. These results suggest an enhanced MV to decreased intraluminal pressure in the pregnant state due to NO. This potential role for NO in pregnancy may promote greater effectiveness of CBFAR during hypotension.

THE MATERNAL BLOOD-BRAIN BARRIER PERMEABILITY IN PREECLAMPSIA: INVOLVEMENT OF LECTIN-LIKE OXIDIZED LOW-DENSITY LIPOPROTEIN RECEPTOR-1 AND PEROXYNITRITE GENERATION

Malou P Schreurs, M.D, Carl A Hubel, Ph.D, Ira M Bernstein, M.D. and Marilyn J Cipolla, Ph.D
Dept. of Neurology, UVM

Preeclampsia is a hypertensive multisystem disorder with endothelial dysfunction as its centre. Neurological complications resulting from blood-brain barrier (BBB) disruption do not occur in all preeclamptic women, suggesting different levels of severity. In addition, oxidative stress, particularly peroxynitrite ($ONOO^-$), is thought to be important in the occurrence of systemic endothelial dysfunction in preeclampsia. Also, lectin-like oxidized low -densitiy lipoprotein receptor-1 (LOX-1) expression, an enhancer of oxidative stress by increasing $ONOO^-$ generation, is elevated in preeclampsia. However, the involvement of LOX-1 and $ONOO^-$ generation in disrupting the BBB is not known. Using a rat model, we measured the influence of plasma from normal pregnant, mild preeclamptic (MPE) and severe preeclamptic (SPE) women on BBB permeability compared to nonpregnant (NP) plasma, and examined the influence of LOX-1 and $ONOO^-$ generation on BBB permeability in preeclampsia. Hydraulic conductivity, a measure of BBB permeability, was compared. Both MPE and SPE plasma significantly increased BBB permeability compared to NP plasma ($p<0.05$). However, only SPE plasma significantly increased BBB permeability compared to all other groups, including MPE plasma ($p<0.01$ vs. all). Either FeTMPyP ($ONOO^-$ scavenger) or a LOX-1 antibody prevented the increase in BBB permeability induced by SPE plasma ($p<0.01$). Our findings suggest that circulating factors present in SPE plasma increase BBB permeability by increasing the activity of LOX-1 and increased generation of $ONOO^-$, which may explain the increased risk of BBB disruption in this group.

THE EFFECTS OF VOLUNTARY EXERCISE ON DISCRIMINATION AND SET-SHIFTING IN MALE WISTAR RATS

Meghan C. Eddy, Jessica N. Savrann, Katharine M. Rifken, Samantha M. Luce, and John T. Green
Department of Psychology, The University of Vermont

Most previous animal research has focused on exercise-related changes in the hippocampus. The effect of exercise on learning that requires other brain regions, such as the prefrontal cortex and striatum, has been largely unstudied. Here we looked at the effects of voluntary exercise on the ability of rats to discriminate rewarded from unrewarded arms in a T maze based on one stimulus dimension of the arms and their ability to set-shift, in which they have to discriminate based on another stimulus dimension of the arms. The initial discrimination requires frontal sensorimotor cortex and dorsolateral striatum while the set-shift requires medial prefrontal cortex and dorsomedial striatum. Rats were given access to running wheels for two weeks prior to testing. In set 1, rats were trained to criterion. In set 2, the rats were required to make a set-shift. Exercising rats were better at the initial discrimination but did not show an enhanced ability to set-shift. Dopamine transporter (DAT) levels from striatum tissue of rats used in the first experiment were examined using Western blots, and preliminary results indicate that exercising rats have less DAT protein expression. We are examining the dorsolateral striatum (DLS) as one of the substrates for the beneficial effects of exercise. Exercising rats appeared to be less susceptible to disruptions caused by the D1 antagonist, possibly due to a down regulation of D1 receptors in the DLS. Preliminary data from infusions of the D2 antagonist indicate that it may inhibit the performance of exercisers.

GENETICS OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS SUPPORTS THE ROLE OF T HELPER CELLS IN MULTIPLE SCLEROSIS PATHOGENESIS

Elizabeth P. Blankenhorn[1], Russell Butterfield[2], **Laure K. Case[3],** Emma H. Wall[3], Roxana del Rio[3], Sean A. Diehl[3], Dimitry Krementsov[3], Naresha Saligrama[3], and Cory Teuscher[3]

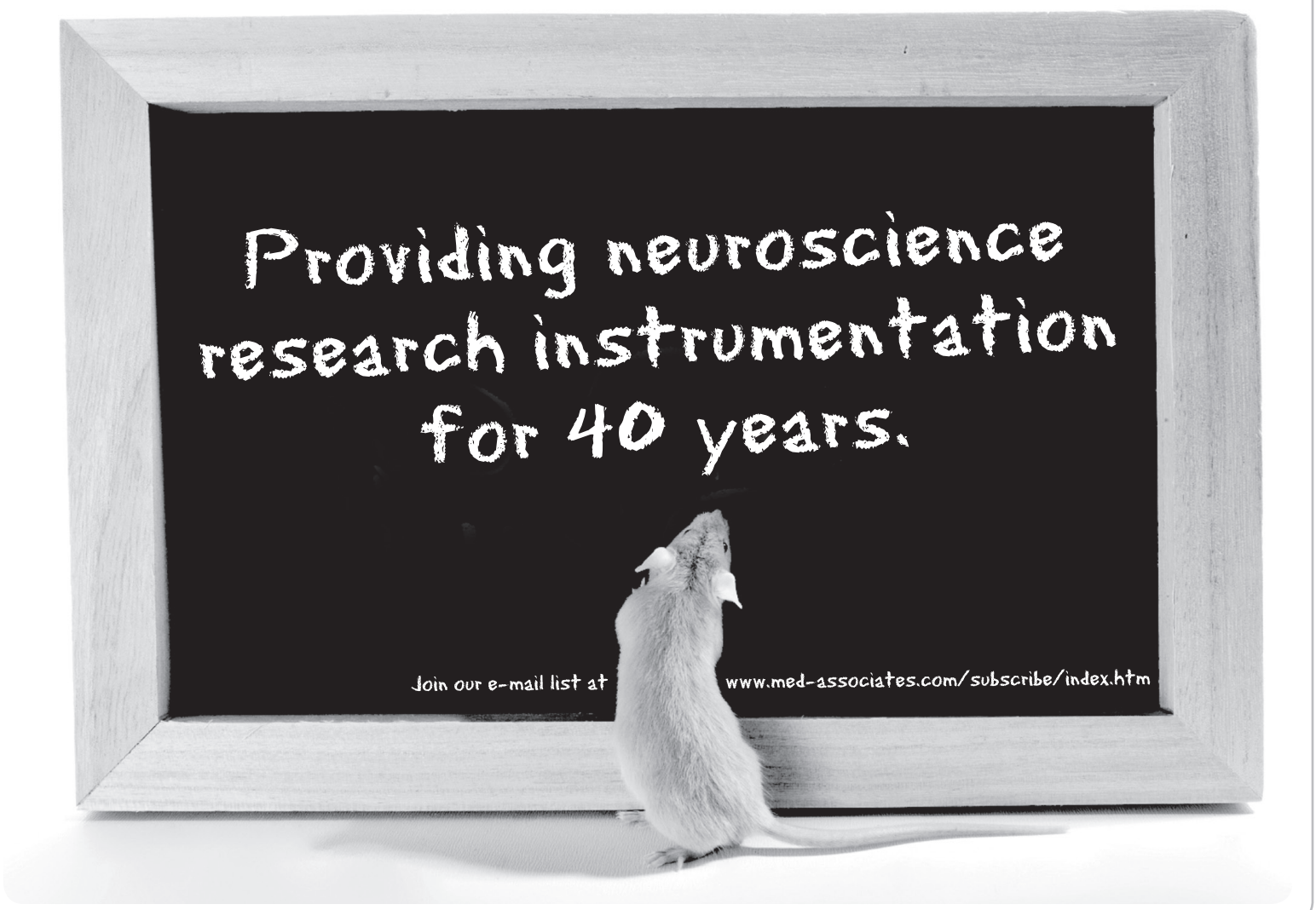
[1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, USA; [2]Departments of Neurology and Pediatrics, University of Utah, Salt Lake City, UT, USA; [3]Department of Medicine and [4]Department of Pathology, University of Vermont, Burlington, VT, USA

Objective: The major histocompatibility complex (MHC) is the primary genetic contributor to multiple sclerosis (MS) and experimental allergic encephalomyelitis (EAE), but multiple additional interacting loci are required for genetic susceptibility. The identity of most of these non-MHC genes is unknown. In this report, we identify genes within evolutionarily conserved genetic pathways leading to MS and EAE.

Methods: To identify non-MHC binary and quantitative trait loci (BTL/QTL) important in the pathogenesis of EAE, we generated phenotype-selected congenic mice using EAE-resistant B10.S and EAE-susceptible SJL mice. We hypothesized that genes linked to EAE BTL/QTL and MS-GWAS can be identified if they belong to common evolutionarily conserved pathways, which can be identified with a bioinformatic approach using Ingenuity software.

Results: Many known BTL/QTL were retained and linked to susceptibility during phenotype selection, the most significant being a region on chromosome 17 distal to *H2* (*Eae5*). We show in pathway analysis that T helper (T_H)-cell differentiation genes are critical for both diseases. Bioinformatic analyses predicted that *Eae5* is important in CD4 T-effector and/or Foxp3$^+$ T-regulatory cells (Tregs), and we found that B10.S-*Eae5*^{SJL} congenic mice have significantly greater numbers of lymph node CD4 and Tregs than B10.S mice.

Interpretation: These results support the polygenic model of MS/EAE, whereby MHC and multiple minor loci are required for full susceptibility, and confirm a critical genetic dependence on CD4 T_H-cell differentiation and function in the pathogenesis of both diseases.



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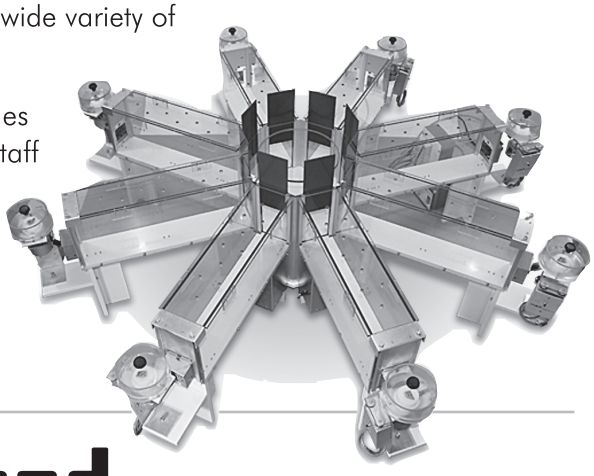

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Abstracts

Posters

EXPRESSION AND FUNCTION OF CCL2/CCR2 IN URINARY BLADDER WITH CYCLOPHOSPHAMIDE (CYP)-INDUCED CYSTITIS IN FEMALE RATS

L. Arms, B.M. Girard, S. Malley, K. Corrow, M.A. Vizzard
University of Vermont College of Medicine, Department of Neurology and Anatomy and Neurobiology, Burlington, VT 05405

Bladder Pain Syndrome/Interstitial Cystitis is a chronic bladder pain syndrome with symptoms of urgency, frequency, nocturia, suprapubic and pelvic pain. While the etiology of BPS/IC is unknown, we hypothesize that pro- inflammatory molecules including, but not limited to, chemokines, chemotactic cytokines, may contribute to bladder sensory dysfunction. The goal of the current studies was to examine the expression and role of a chemokine/receptor pair, CCL2/CCR2 in the urinary bladder following cyclophosphamide (CYP)-induced cystitis. We induced bladder inflammation in adult female Wistar rats (200-300g) by injecting CYP intraperitoneally at acute (150 mg/kg; 4h), intermediate (150 mg/kg; 48h) and chronic (75 mg/kg; every third day for 8 days) time points. CCL2 protein and mRNA expression in the urinary bladder was significantly ($p \leq 0.01$) increased with 4h and 48h CYP treatment as demonstrated with ELISAs and Q-PCR. CCR2 mRNA was significantly ($p \leq 0.01$) increased in the urothelium in the detrusor smooth muscle with 4h and 48h CYP-treatment, respectively. CCR2-immunoreactivity was significantly ($p \leq 0.01$) upregulated in all layers of the urothelium following 4h CYP treatment. In rats with 4h CYP-induced cystitis, intravesical administration of RS504393, a CCR2 receptor antagonist, significantly ($p \leq 0.01$) increased bladder capacity (2-3 fold) and increased the duration of the intercontraction interval. These data demonstrate that the expression of CCL2/CCR2 changes in the urinary bladder with inflammation and that blockade of CCL2/CCR2 interactions can improve urinary bladder function in CYP-induced cystitis. Support: DK051369, DK060481, DK065989 and P20RR16435

KNOCKDOWN OF SCN1A IN THE MS/VDB ALTERS HIPPOCAMPAL THETA AND IMPAIRS SPATIAL RECOGNITION MEMORY IN RATS

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Objective: Dravet syndrome (DS) is a childhood-onset epileptic encephalopathy caused by mutations in *SCN1A* that result in loss of function of the type I voltage-gated sodium channel ($Na_v1.1$). Although cognitive impairment in DS has been attributed to seizures, etiology may also independently contribute to outcome. The finding in DS mouse models that fast neuronal firing is affected suggests that the temporal coordination of brain oscillations and information processing are likely to be impaired by $Na_v1.1$ loss of function. We hypothesized that a loss of function of $Na_v1.1$ in regions critical for the coordination of brain oscillations would disrupt cognitive function.
Methods: To investigate this hypothesis, we used *in vivo* RNA interference to knockdown expression of *SCN1A* in the medial septum and ventral diagonal band of Broca (MSvDB), a region that is required for the coordination of theta (5-12 Hz) oscillations in the hippocampus and for spatial memory. We subsequently tested performance on a hippocampal-dependent, response-to-novelty task.
Results: We found that knockdown of $Na_v1.1$ in the MSvDB impairs spatial recognition memory but not object identity recognition, consistent with the role of the MSvDB and hippocampus in spatial cognition. Performance was associated with reduced hippocampal theta frequency that paralleled the spatial memory impairment. Continuous EEG monitoring indicated that this effect is not caused by the occurrence of seizures.
Interpretation: These results suggest a critical role for $Na_v1.1$ in the MSvDB. More broadly, it suggests that Nav1.1 is required for normal cognitive function. Therefore, $Na_v1.1$ deficits in DS are likely to independently contribute to cognitive impairment, possibly through a dysregulation of oscillations. New therapeutic approaches targeting such mechanisms could be beneficial to patients.

MORPHOLOGY OF THE CHOLINERGIC SYSTEM IN THE CNS OF LYNX1 AND LYNX2 NULL MICE: AN IMMUNOHISTOCHEMICAL STUDY

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The cholinergic system comprised of projections from neurons in the basal forebrain to cortex and hippocampus contributes to cognition, memory, and attention. Of particular interest are cholinergic responses mediated by nicotinic acetylcholine receptors (nAChRs), which modulate neurotransmitter release in the brain. For example, nicotine treatment of individuals with impaired cognition improved performance on attentional memory tasks (Newhouse et al. 2004, Curr Opin Pharmacol 4:36). More recently, a new class of endogenous neurotoxin-like molecules has been discovered that bind and alter the kinetics of nAChRs. Mice lacking two of these proteins, lynx1 and lynx2, have been generated and both lines display enhanced nicotinic responses in the CNS and distinct behavioral phenotypes (Miwa et al 2006. Neuron 51:587; Tekinay et al 2006. PNAS 106:4477). We hypothesize that the loss of lynx1 and/or lynx2 causes changes in the cholinergic basal forebrain system. Brains of wild-type, lynx1 KO and lynx2 KO male mice, were harvested, immersion fixed and serially-sectioned. Tissue was immunostained to visualize choline acetyltransferase (ChAT) using horseradish peroxidase. The density of ChAT-positive terminals and number of cell bodies in the basal forebrain cholinergic nuclei were quantified using a video-based, computer-assisted analysis system (MBF Bioscience, Williston, VT). Lynx1 KO and lynx2 KO brains contained less ChAT-stained cell bodies in the diagonal band and medial septum compared to wild type controls. Labeling with control neural marker anti-$p75^{NTR}$ showed fewer neurons in lynx1 KO compared to wild type but no difference in lynx2 KO in the same regions. Lynx1 KO, but not lynx2 KO, expressed lower density of ChAT-positive terminals in cortex. The decrease in p75-positive neurons in diagonal band and medial septum of lynx1 KO suggests presence of lynx1 contributes to survival of cholinergic neurons in basal forebrain. Lower expression of axon terminals in lynx1-KO supports the hypothesized model of a compensatory down-regulation of cholinergic transmitter synthesis. Tracking these morphological changes are critical in our understanding of the complex regulatory mechanisms of prototoxin genes and will further elucidate a model for cognitive disorders linked to cholinergic activity.

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CONTEXT-DEPENDENT TRKB SIGNALING PROMOTES DIFFERENTIATION OR TRANSFORMATION IN CELL LINES OF THE NEURAL CREST LINEAGE

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Neuroblastoma is a common pediatric malignancy that arises in the developing sympathetic nervous system, a structure derived from the neural crest. In neuroblastoma, expression of the neurotrophin receptor TrkB and its ligand, brain-derived neurotrophic factor (BDNF) is predictive of a poor prognosis and a more aggressive form of disease. Although the oncogenic potential of activated TrkB signaling has been demonstrated in established neuroblastoma cell lines, the ability of TrkB signaling to transform the non-cancerous developmental precursors from which neuroblastoma arises has not been investigated. In order to further understand the role of TrkB signaling in transformation of the developing sympathetic nervous system in neuroblastoma we have created a constitutively active form of the TrkB receptor (ΔIgTrkB) by removal of the two immunoglobulin-like ligand binding domains. This construct is expressed at the cell surface and is constitutively active as shown by phosphorylated Erk 1/2 activation. Interestingly, when transfected into PC12 cells constitutive TrkB signaling promoted process outgrowth. Conversely, when ΔIgTrkB was expressed in the neural crest derived cell line NCM-1, TrkB signaling led to the development of a markedly transformed and metastatic phenotype characterized by increased proliferation and the ability for anchorage independent cell growth in soft agar. Furthermore, expression of ΔIgTrkB led to upregulation of a number of oncogenes, as well as downregulation of tumor suppressors. These results indicate that unregulated TrkB signaling has the ability to promote a tumorogenic phenotype in the proper context, such as the neural crest, and suggests that activated TrkB signaling may be sufficient to promote neuroblastoma formation in the developing sympathetic nervous system.

NICOTINIC AGONIST MODULATES PERFORMANCE MONITORING ON THE STOP SIGNAL TASK IN ADULTS WITH ADHD

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People with Attention-Deficit/Hyperactivity Disorder (ADHD) have deficits in executive functions, including response inhibition and performance monitoring (PM). Deficits in response inhibition, as measured by the Stop Signal Task (SST) can be modified through nicotinic stimulation. PM relies on attentional orienting and signal detection, also modulated by the nicotinic cholinergic system. We hypothesized that AZD3480, an α4β2 nicotinic agonist, would improve PM during the SST in adults with ADHD.

This was a secondary analysis of a study (NCT00683462) to determine the safety and efficacy of AZD3480 in adult ADHD. 24 non-smokers with ADHD participated in a 3-way cross-over double-blind trial including randomized 2-week periods of dosing (AZD3480 5 mg, 50 mg, and placebo), separated by washout. PM during the SST was measured by changes in reaction time after stop versus go trials. Mixed model ANOVAs were used to analyze the difference in slowing of post-stop trials across drug conditions.

Treatment with AZD3480 significantly slowed post-stop trials. Specifically, trials following failed inhibition, post-error trials, showed significant slowing while those following successful inhibition did not. Further analysis showed a treatment effect, with the greatest slowing at the 50 mg dose.

This study is the first to suggest that PM, through post-error adjustments, may be modulated by nicotinic stimulation in ADHD. Slowing was not associated with the mere presence of the stop signal, but rather was associated specifically with failed inhibition. These results demonstrate that PM can be studied using the SST in ADHD, and that performance is modulated by nicotinic cholinergic system function.

THE EFFECTS OF METHYLMERCURY ON THE NOTCH SIGNALING PATHWAY AND MOTOR NERVE FORMATION DURING DROSOPHILA EMBRYONIC DEVELOPMENT

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Methylmercury (MeHg) is a harmful and ubiquitous toxicant that provokes neurological defects in the developing fetal nervous system. MeHg has been shown to target the Notch pathway, a highly-conserved intercellular signaling mechanism responsible for normal development and neurogenesis. We have previously shown in a *Drosophila* model system that MeHg significantly upregulates a number of the conventional Notch target genes of the *Enhancer of Split* (*E(spl)*) complex. Upregulation of *E(spl)mδ* can be seen consistently *in vitro* in neural-derived cell lines and *in vivo* in whole embryos. We have recently observed that MeHg treatment in embryos causes branching failure in the segmental nerve (SN), a motor nerve readily observed using immunostaining. This SN phenotype is replicated in embryos where *E(spl)mδ* is artificially overexpressed in muscle. This indicates that overexpression of *E(spl)mδ* in the target tissue of the developing SN may be the mechanism by which MeHg inhibits branching. Overall, these data confirm that the ability of MeHg to augment signaling in the Notch pathway may be key to some aspects of its toxicity.

EXPRESSION AND PLASTICITY OF CORTICOTROPIN RELEASING FACTOR (CRF) AND RELATED PEPTIDES/RECEPTORS IN URINARY BLADDER DURING POSTNATAL DEVELOPMENT IN RAT

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CRF is prominently expressed in central and peripheral micturition pathways including the descending pathway from Barrington's nucleus to and within the sacral parasympathetic nucleus. During the course of postnatal maturation, a spinobulbospinal reflex leading to emergence of voluntary voiding replaces primitive reflex pathways organized at the spinal level. Postnatal maturation of voiding function may involve prominent reorganization of synaptic connections in bladder reflex pathways in the brain, spinal cord, and periphery. Previous studies have suggested the importance of neuroactive compounds in the process of maturation of the micturition reflexes during prenatal and early postnatal development. These changes in micturition reflexes during postnatal development may be mediated, in part, by changes in the neurochemical properties, including the CRF and related peptides/receptors, of central micturition pathways as well as by similar changes in the urinary bladder. In this study we examined: (1) urocortin I-III mRNA expression and CRF receptor (R) 1 and CRFR2 expression in urinary bladder (urothelium and detrusor smooth muscle during early postnatal development (P1-P36). Ongoing studies are examining CRF and urocortin I-III and CRF receptor expression in other components of the micturition reflex including lumbosacral spinal cord including the sacral parasympathetic nucleus and dorsal root ganglia. Changes in neuroactive compounds in central and peripheral aspects of the micturition reflex may underlie postnatal maturation of voiding reflexes.

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EXPRESSION AND REGULATION OF NEUROPEPTIDES/RECEPTORS IN MICTURITION REFLEX PATHWAYS OF NERVE GROWTH FACTOR (NGF) OVEREXPRESSING (OE) MICE WITH CYCLOPHOSPHAMIDE (CYP)-INDUCED CYSTITIS

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Our previous studies have demonstrated expression and regulation of PACAP, PAC1, VPAC1, VPAC2 transcripts in urinary bladder and lumbosacral dorsal root ganglia with CYP-induced cystitis. Enhanced target-derived NGF availability increases PACAP expression in small nociceptive DRG cells. NGF may play a role in urinary bladder dysfunction by mediating inflammation and functional changes in sensory and sympathetic neurons innervating the urinary bladder. We have also previously demonstrated NGF regulation of PACAP/receptors in bladder reflex pathways using a transgenic mouse model of chronic NGF overexpression in the bladder using the urothelial-specific uroplakin II (UPII) promoter. In this study, we have explored the contribution of target-derived NGF in combination with CYP-induced cystitis to determine if additional changes in neuropeptides/receptors would be observed in micturition reflex pathways due to the presence of additional inflammatory mediators in the urinary bladder. NGF was over-expressed at the mRNA and protein level in the bladders of transgenic compared to WT littermate control mice. Transgenic mice had a reduced bladder capacity in conscious open-voiding cystometry studies and an increased pelvic hypersensitivity. Quantitative PCR was used to determine PACAP/VIP, substance P, galanin and receptor transcripts expression in the urinary bladder (urothelium, detrusor smooth muscle) in NGF-OE and WT mice with CYP-induced cystitis (4 hour (h), 48 h, chronic). We are currently determining if additional changes in neuropeptide and receptor mRNA expression are associated with immunoreactivity and functional changes. These studies are consistent with target-derived NGF and other inflammatory mediators affecting neurochemical plasticity and reflex function of micturition pathways.

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COMPARATIVE PHOSPHOPROTEOMIC ANALYSIS OF NEONATAL AND ADULT MURINE BRAIN

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Developmental processes are governed by diverse regulatory mechanisms including a suite of signaling pathways employing reversible phosphorylation. With the advent of large-scale phosphoproteomics it is now possible to identify thousands of phosphorylation sites from tissues at distinct developmental stages. We describe here the identification of over 6,000 non-redundant phosphorylation sites from neonatal murine brain. When compared to nearly three times the number of phosphorylation sites identified from three-week-old murine brain, remarkably one-third of the neonatal sites were unique. This fraction only dropped to one-quarter when allowing the site to stray plus or minus 15 residues. Using quantitative mass spectrometry we characterized a novel phosphorylation site (Ser265) identified uniquely in the neonatal brain on Doublecortin (Dcx), a protein essential for proper mammalian brain development. While the relative levels of Dcx and phospho-Ser265 Dcx between embryonic and neonatal brain were similar, their levels fell precipitously by postnatal day 21, as did phospho-Ser297, a site required for proper neuronal migration. Both sites lie near the microtubule-binding domain and may provide functionally similar regulation via different kinases. These data provide evidence for considerable change in the profiles of developmentally-regulated phosphoproteomes. Toward a more global characterization of the changes of proteomes and phosphoproteomes across brain development, we also present emerging data from a quantitative proteomics experiment comparing embryonic and adult murine brain.

GABAA RECEPTOR-MEDIATED TONIC CONDUCTANCE IN NEURONS OF THE MOUSE MEDIAL SEPTUM/DIAGONAL BAND OF BROCA (MS/DB)

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In most brain regions, GABA-mediated inhibition consists of a "phasic" component due to moment-to-moment activation of GABAA receptors at synaptic sites and a "tonic" component due to sustained activation of extrasynaptic GABAA receptors by ambient GABA. Previously we reported that nerve growth factor (NGF) increases neuronal excitability and attenuates GABAA receptor-mediated synaptic transmission in cholinergic neurons of the medial septum and diagonal band of Broca (MS/DB). We hypothesized that tonic GABA conductance is a potential target for NGF modulation as well. However, whether extrasynaptic GABAA receptors and tonic GABA conductance are functional in the MS/DB has not been demonstrated. Therefore, we initiated a study to establish their presence and, if so, whether NGF could modulate tonic GABAA receptor-mediated conductance in the MS/DB.

Patch clamp recordings were performed in cholinergic and non-cholinergic MS/DB neurons identified in 200-m acute coronal brain slices from P6-P20 C57BL/6 mice. The average amplitude of tonic GABA current was 5.11±0.79 pA with a KCl pipette solution and 2.70±0.37 pA with a K-gluconate pipette solution, with no significant difference across ages or between cell-types. Using either single-cell or whole-septum RT-PCR, we detected transcripts encoding different GABAA receptor subunits, including α5, γ2, and δ. Western blot analysis of whole septum homogenates from P20 C57BL/6 mice also revealed the presence of the GABAA receptor δ subunit. Double immunohistochemical labeling revealed δ subunit expression in the majority of VAChT-immunopositive (cholinergic) and in some parvalbumin-immunopositive (GABAergic) MS/DB neurons.

We characterized pharmacologically the functional subunit composition of extrasynaptic GABAA receptors in the MS/DB using GABAA receptor subunit-selective modulators. THIP (1μM), a selective agonist for δ-containing extrasynaptic GABAA receptors, induced a small inward shift in the holding current (1.40±0.44 pA). L-655,708 (5μM), the selective inverse agonist at α5-containing extrasynaptic GABAA receptors, caused an outward shift (-6.82±2.52 pA) in the holding current. Diazepam (0.5-1μM) caused a small inward shift (1.17±1.12 pA) in the holding current.

Taken together, these results show that extrasynaptic GABAA receptors in the MS/DB are functional; they likely consist of α5-containing receptors, and to a lesser extent δ subunit-containing receptors. Ongoing work continues to explore NGF's effect on tonic GABA conductance and neuronal excitability.

PRIOR STRESS INTERFERES WITH THE ANXIOLYTIC EFFECT OF VOLUNTARY EXERCISE IN C57BL/6J MICE

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We have shown that voluntary exercise in mice is associated with reduced anxiety across several anxiety models. Recent studies suggest that social isolation stress may delay or prevent the anxiolytic effect of voluntary exercise. To evaluate this possibility, C57BL/6J mice were subject to social isolation and to a chronic variate stress procedure in which mice were exposed to one of 5 different stressors each day for 7 days. Control mice were group housed and handled daily over 7 days. Following stress exposure, separate groups of stress and control mice were given either functioning or non-functioning (i.e., locked) running wheels for three weeks. The anxiolytic effect of exercise was assessed using acoustic startle, stress-induced hyperthermia and a challenge with the anxiogenic drug mCPP. Consistent with numerous reports, stress interfered with normal weight gain. There were no differences in the average distance stress and control mice ran in each 24 hr period. Despite three weeks of wheel running, exercise was not anxiolytic in stress mice. Compared to control-sedentary mice, control-exercising mice showed reduced acoustic startle amplitude, reduced stress-induced hyperthermia and a blunted anxiogenic effect of mCPP. Stressed-exercising mice, on the other hand, were indistinguishable from stressed-sedentary mice on each measure of anxiety and both stressed groups were similar across measures to the control-sedentary mice. Although average running distance varied considerably across individual stress and control mice, running distance did not predict the level of anxiety on any measure. These results suggest that prior and ongoing stress delays or prevents the anxiolytic effect of exercise without affecting exercise itself.

STEP INITIATION WITH MULTIPLE SCLEROSIS: EFFECTS OF STANCE WIDTH, CUEING, AND DUAL TASKING

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Objective: To identify the effects of multiple sclerosis (MS) on voluntary step initiation.
Method: Thirteen subjects with MS and 13 matched subjects without MS performed trials of step initiation in a series of conditions to evaluate (1) self-initiated stepping at narrow (6% body height) versus standard (11% body height) stance widths, (2) self-initiated versus cued stepping, and (3) stepping with and without performing an auditory stroop task. The timing and amplitude of the anticipatory postural adjustment (APA) as well as the timing, amplitude and velocity of the step were evaluated with two-factor ANOVA comparing the two groups and the paired conditions above.
Results: Subjects with and without MS exhibited similar APA and step characteristics from a narrow stance width as well as similar scaling of APA amplitudes and step velocities across narrow to standard stance widths. At standard stance widths, with and without cueing, the subjects with MS exhibited longer APA durations than subjects without MS. With increasing disease severity, the APA duration increased, the ability to modify APA duration with cueing decreased, and the step velocity decreased. Lastly, during cued stepping with and without dual tasking, the subjects with MS exhibited increased APA durations with subsequently delayed foot-lift latencies. The stroop task delayed the posterior component of the APA more for the subjects with MS than for those without MS, and the stroop task associated with increased step lengths for subjects with MS but decreased step lengths for those without MS. During the dual-task condition, step velocities decreased and foot-lift latencies increased with increasing disease severity.
Conclusion: MS impairs the time spent in postural preparation and foot-swing velocity, particularly with disease progression, as well as the ability to initiate stepping under dual-task conditions.
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FUNDAMENTAL CHANGE IN NEUROVASCULAR COUPLING AFTER SUBARACHNOID HEMORRHAGE

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Subarachnoid hemorrhage (SAH) following cerebral aneurysm rupture is associated with substantial morbidity and mortality against which existing therapeutic options have limited efficacy. The impact of SAH on neurovascular coupling, reflecting the coordinated communication between neurons, astrocytes and parenchymal arterioles, is unknown. Using a combination of two-photon and infrared-differential interference contrast microscopy, arteriolar diameter and astrocyte endfoot Ca^{2+} were simultaneously measured in brain slices from un-operated, sham-operated and SAH model of rats. Increased neuronal activity caused by electrical field stimulation (EFS) elicited the predicted elevation in endfoot Ca^{2+} and vasodilation in brain slices from control animals (Filosa JA et al, 2006). EFS induced a similar increase in endfoot Ca^{2+}, but in marked contrast caused parenchymal arteriolar constriction in brain slices from SAH model animals. Further, elevating endfoot Ca^{2+} via two-photon photolysis of caged Ca^{2+} in the range of 200-500 nM caused parenchymal arteriolar dilation in brain slices from control animals and constriction in brain slices from SAH animals. These data demonstrate that SAH causes a fundamental switch from vasodilation to constriction in response to moderate increases in astrocyte endfoot Ca^{2+}.
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DIFFERENTIAL REGULATION OF PACAP/PAC1R EXPRESSION BY CORTICOSTERONE AND STRESS

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Repeated variate stress results in a multitude of behavioral and neurochemical changes in areas implicated in anxiety and stress responding. Following exposure to repeated variate stress, pituitary adenylate cyclase activating polypeptide (PACAP) and PAC1 receptor transcript levels are substantially increased in the bed nucleus of the stria terminalis (BNST), an area heavily implicated in anxiety-like behavior and stress-responding. The mechanisms through which these changes occur, including the role of stress-induced elevations in circulating glucocorticoids, are still unclear. In the current set of studies, male rats were subjected to a single stress exposure, 7 days of repeated variate stress, or no stress. Micropunches of specific brain areas were examined for differences in transcript expression of PACAP and PAC1 receptor using quantitative PCR. PAC1 receptor transcript levels were increased only after repeated stressor exposure within the dBNST. Repeated and single stressor exposure, increased PACAP transcript expression within the dBNST. To determine the role of glucocorticoids in mediating these changes male rats were administered either a single or 7-days treatment of subcutaneous corticosterone (cort) or equivolume vehicle. Neither repeated nor single cort treatment produced an increase in PAC1 receptor expression. While repeated stressor exposure increased PACAP transcript levels within the dBNST in 5/6 rats, repeated cort treatment only produced this increase in 3/10 rats. These data suggest that elevated cort may interact with other variables to promote BNST PACAP signaling. These studies further adds to the knowledge regarding the role of stressor exposure and stress hormone exposure on PAC1 and PACAP expression.

KEYWORDS: STRESS, BED NUCLEUS OF THE STRIA TERMINALIS, CORTICOSTERONE

INTER-MUSCULAR PATTERNS OF MUSCLE ACTIVATION FOLLOWING 10-WEEK TREATMENT FOR CHRONIC LOW BACK PAIN

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Purpose/Hypothesis: Chronic low back pain (LBP) is associated with altered automatic postural responses (APR) that are thought to reflect deficits in the strength and control of abdominal muscles [1]. Clinical interventions that target these muscle impairments have been promoted; however, the treatment effects on muscle activation impairments remain unclear. This study evaluated the effects of two treatments (trunk stabilization vs. general strength and conditioning) on muscular patterns of EMG onsets in persons with LBP.
Number of Subjects: 39 subjects with recurrent LBP (i.e., longer than six months)
Material/Methods: Subjects were randomly assigned to one of two, 10-week treatments: stabilization (STB) (n = 22, 9 female, 40(9) years) or general strength and conditioning (STC) (n = 17, 11 female, 41 (8) years). Pre- and post-treatment, EMG data from the trunk and left lower limb were recorded using surface electrodes. Subjects stood and were randomly presented with 3 support surface translations in each of 12 horizontal directions. A mixed model repeated measures ANOVA, with fixed effects of treatment group (STB and STC), perturbation direction, and treatment visit (pre- and post-treatment) was used to determine differences in the1) percent of trials with bursts of EMG onsets in early and late phases of the APR, and 2) amplitudes of integrated EMG pre- and post-perturbations. Post-hoc analyses for significant interactions ($p < 0.05$) were performed using the least squares means method.
Results: There were no differences in group demographics and both groups showed similar improvements in Numeric Pain Rating Scale, Oswestry Disability Index, and McGill Pain Questionnaire. Post-treatment, both groups had a lower percentage of trials with EMG bursts in the early phase for the internal oblique, while the STB group also had fewer bursts in the medial gastrocnemius. Also, the percentages of trials with EMG bursts in the late-phase were reduced in the trunk muscles of the STB group and in the tibialis anterior of the STC group. The amplitude of the EMG post-treatment during the baseline period was increased for the medial gastrocnemius, abdominal and back muscles across both groups post-treatment. Post-treatment, abdominal muscle amplitudes following surface translations tended to increase in both groups and these increases were largest in the STC group.
Conclusions: Despite two different treatments, the number of both early- and late-phase EMG bursts tended to decrease and the magnitude of muscle activation increased, particularly in the abdominal muscles for both groups. Clinical improvements were also similar in both groups.
Clinical Relevance: The results indicate that the STB treatment protocol does not preferentially improve treatment outcomes or inter-muscle coordination patterns for persons with LBP.
References: [1] Cholewicki, et al. 2005. Spine 30(23): 2614-2620.

CHARACTERIZATION OF ASPARTOACYLASE IN OLIGODENDROGLIOMA

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It is well accepted that the metabolism of proliferating and differentiated cells differs. However, little attention has been focused on N-acetyl-L-aspartate (NAA), one of the most abundant amino acid derivatives in the brain, or its synthetic product, N-acetyl-aspartyl-glutamate (NAAG). NAA and aspartoacylase (ASPA), the enzyme responsible for NAA degradation, are significantly reduced in oligodendroglioma tumors, suggesting a tumor suppressive role. This study sought to characterize the effects of NAA and NAAG on oligodendroglioma and oligodendrocyte precursor cell (Oli-neu) proliferation and differentiation. NAA and NAAG had no effect on the proliferation of established cell lines (Hs638, HOG, Oli-neu), but significantly increased proliferation of oligodendroglioma stem-like cells (OG33, OG35), refuting a tumor suppressor role for these molecules. Differentiation of Oli-neu cells with cAMP down-regulated ASPA expression, which could be rescued by NAA treatment. This regulation is likely due to a cAMP-mediated signaling pathway rather than differentiation since Oli-neu differentiation induced with the ErbB2 antagonist PD174265 had no effect on ASPA expression. NAA and NAAG treatment had no effect on ASPA expression in Hs683 and HOG cells, but significantly up-regulated ASPA expression in OG33 and OG35 cells. ASPA was prominently expressed in the nuclei in oligodendroglioma stem-like cells suggesting a possible role for ASPA and NAA metabolism in this subcellular compartment. Collectively, these results identify oligodendroglioma stem-like cells as being uniquely receptive to alterations in NAA and NAAG levels and sets the stage for investigations into potential therapeutics that target pathways involved in NAA and NAAG metabolism in the treatment of oligodendroglioma.

DISRUPTION OF TRKB PREVENTS DRG AXON OUTGROWTH IN E5 CHICKEN EMBRYO

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Dorsal root ganglia (DRG) are neural crest derived structures responsible for transmitting sensory information from peripheral sensory receptors to the spinal cord. During neural development, the central processes of DRG cells extend into the spinal cord at the dorsal root entry zone in a three step process: axons extend toward and into the cord on embryonic day 3 (E3), branch rostrally and caudally to establish a longitudinal pathway (E4-6) and finally enter the grey matter (E8). The rostrocaudal extension of sensory afferents is essential for the establishment of intersegmental communication that enables appropriate perception and reflexes, as well as establishing relationships with supraspinal centers.

Our laboratory is interested in the regulation of longitudinal growth of sensory axons in the developing spinal cord. We have shown that axon extension in the longitudinal pathway is significantly reduced by inhibition of myosin II and by blocking β1 integrin receptor with a function blocking antibody. Brain-derived neurotrophic factor (BDNF) has been shown to promote survival and regeneration of sensory afferents, and is known to interface with the cytoskeleton working through its cognate receptor, tyrosine receptor kinase B (TrkB). The current experiments are designed to test the hypothesis that TrkB plays a role in the rostrocaudal extension of sensory afferents in the developing spinal cord.

Immunohistochemistry showed expression of TrkB immunoreactivity from E4 to E8 in the longitudinal pathway of the thoracic spinal cord. To determine whether TrkB is functionally involved in sensory afferent growth, thoracic level 4 (T4) DRG of 100-hour chicken embryos (stage-25) were micro-injected with DiI in an in vitro preparation of the ganglia and spinal cord. Preparations were then cultured for 5-hours in the presence of a TrkB inhibitor, K252a or vehicle control. DiI labeled sensory afferents were measured using multiphoton microscopy and Volocity software. Significant truncation ($P<0.001$) of longitudinally extending axons was observed in K252a treated embryos compared to vehicle controls. A TrkB function blocking antibody was then used to achieve a more specific inhibition of the TrkB receptor. Significant trunctation ($P<0.001$) of extending axons was observed upon application of the TrkB antibody compared to IgG control treatment. Collectively, these data suggest a role for TrkB in axon outgrowth during development of the longitudinal pathway.

EFFECTS OF PUTATIVE NONSELECTIVE CATIONIC CHANNEL BLOCKERS ON THE PAC1 RECEPTOR-MEDIATED INCREASE IN GUINEA PIG CARDIAC NEURON EXCITABILTY

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Pituitary adenylate cyclase activating polypeptide (PACAP), when released by stimulation of preganglionic nerve fibers or when exogenously applied, significantly increases guinea pig cardiac neuron excitability. The effect is mediated solely by PAC1 receptors as maxadilan, but not vasoactive intestinal polypeptide (VIP), also enhances cardiac neuron excitability. The mechanisms underlying the PACAP-induced modulation of cardiac neuron excitability are not fully elucidated, although enhancement of the hyperpolarization-activated nonselective cationic conductance by cAMP is likely a key contributor. A PACAP-mediated calcium influx also appears to be a critical co-factor, but the pathway for calcium influx is not defined. Extracts of the guinea pig cardiac neurons obtained by laser capture contain transcripts for TRPC 1, 3, 4, and 5. The combination of TRPC 1 and TRPC 4 or TRPC 5 subunits can form a calcium-permeable, nonselective cationic channel and could be the calcium influx pathway. Using intracellular recordings *in vitro* from neurons in atrial whole mount preparations containing the cardiac ganglia, we have tested the effect of three putative nonselective cationic channel blockers on the PACAP-induced increase in cardiac neuron excitability. With bath applied PACAP, approximately 90 percent of the cardiac neurons exhibit a multiple action potential firing pattern when 1 second depolarizing current pulses of increasing strength are applied. In contrast, in the absence of PACAP, approximately 90 percent of the cardiac neurons exhibit a phasic firing pattern with only approximately 10 percent of the cardiac neurons exhibiting a multiple firing pattern. Pretreatment with any of the three putative nonselective cationic channel blockers significantly suppressed the PACAP-induced increase in excitability. However, these drugs did not block the increase in excitability produced by 1 mM barium, suggesting the effect was not due to a nonspecific suppression of excitability. Our results suggest that the PACAP-activated calcium influx pathway likely is a nonselective cationic channel, perhaps a TRPC channel.
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ROLE OF CHRONIC VARIATE STRESS IN BLADDER DYSFUNCTION IN RATS

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Interstitial cystitis (IC)/bladder pain syndrome (BPS) is a chronic pelvic pain disorder characterized by urinary urgency, frequency, and suprapubic pain. All of these symptoms are often exacerbated by stress. The prevalence of micturition disorders is high among people with anxiety disorders, and various stressors often increase levels of anxiety. Acute psychological stress has been shown to activate mast cells in the bladder, which may play a significant role in the pathophysiology of IC/BPS. The aim of the current study was to determine if chronic stress in adult rats, in the absence of any direct insult to the urinary bladder, could change urinary bladder function and referred somatic sensitivity. In addition, due to previous studies demonstrating a role for urinary bladder nerve growth factor (NGF) expression in urinary bladder dysfunction, urinary bladder NGF content was also determined. Stressed rats (n = 8) were exposed to a 7-day, chronic variate stress paradigm, with a single stressor presented on each day. Sham rats (n = 8) were handled but not exposed to stressors. Tubing was implanted into the bladder and bladder function was evaluated with open outlet cystometry. Rats were euthanized and urinary bladder harvested for determining NGF content using ELISAs. Chronic variate stress significantly ($p \leq 0.05$) increased voiding frequency, decreased void volumes and increased somatic sensitivity in the rat hindpaw with all forces of von Frey filaments tested. NGF bladder content significantly ($p \leq 0.05$) increased (4-fold) after chronic variate stress.

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A NEWLY IDENTIFIED PROTOTOXIN, LYPD6B, MODULATES THE FUNCTION OF THE HETEROMERIC NICOTINIC ACETYLCHOLINE ALPHA3 BETA4 RECEPTOR

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Nicotinic signaling through nicotinic acetylcholine receptors (nAChRs) is involved in a variety of neurodevelopmental processes, such as: neural proliferation, differentiation, and survival. A superfamily of proteins known as prototoxins have been identified as proteins involved in the modulation of signaling through nAChRs. In the chicken ciliary ganglion, we have identified a new member of the prototoxin superfamily, LYPD6B. We hypothesize that the newly identified prototoxin LYPD6B binds to the heteromeric alpha3 beta4 nAChR, and as a consequence decreases acetylcholine evoked responses. The end point RT PCR performed from isolated RNA showed LYPD6B to be expressed in ciliary ganglion and brain but not stomach or lung tissues. Furthermore, electrophysiological experiments were performed on two groups of *Xenopus* oocytes. One group expressed an alpha3 beta4 concatemer linked by glutamine linkers, whereas the second group co-expressed the concatemer and LYPD6B. I perfused 200 μm acetylcholine onto the two groups of oocytes, and the oocytes co-expressing alpha3 beta4 and LYPD6B exhibited an averaged decreased response by half when compared to the group expressing the concatemer alone. The results identify LYPD6B to have a neural specific role that is modulating the function of alpha3 beta4. In order to further support the hypothesis that LYPD6B binds to alpha3 beta4, two experiments must be performed: immunocytochemistry (demonstrating co-localization) and a co-immunoprecipitation. However, there is no antibody against the LYPD6B protein. Therefore, a future experiment will involve inserting an epitope tag sequence into the LYPD6B gene.

PAC1 RECEPTORS MEDIATE POSITIVE CHRONOTROPIC RESPONSES TO PACAP27 AND VIP IN ISOLATED MOUSE ATRIA

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The neuropeptides PACAP and VIP have prominent effects on cardiac function in several species, but little is know about their influence on the murine heart. We performed experiments to evaluate the expression of their receptors in mouse heart and to characterize the response of isolated atria to peptide agonists. Quantitative PCR demonstrated that PAC1, VPAC1, and VPAC2 receptor mRNAs are present in the mouse heart. Pharmacological effects of PACAP27, VIP, and the selective PAC1 agonist maxadilan were evaluated in isolated, spontaneously beating atria from C57BL/6 mice. Incremental additions of PACAP27 at 1 min intervals caused a concentration-dependent tachycardia. VIP and maxadilan also caused tachycardia but their potencies were about two orders of magnitude less than that of PACAP27. Increasing the dosing interval to 5 min caused a leftward shift of the concentration-response curve to maxadilan but no changes in the curve for VIP. Under this condition, neither the potency of maxadilan nor its efficacy differed from those of PACAP27. Neither PACAP27 nor maxadilan caused tachyphylaxis, and their effects on heart rate were quite prolonged, even with repeated washing of the tissue. Heart rate occasionally returned to baseline after treatment with PACAP27, but maximal responses to maxadilan were maintained for at least 2h. We conclude that all three VIP/PACAP family receptors are expressed by mouse cardiac tissue, but only PAC1 receptors mediate positive chronotropic responses to PACAP27 and VIP. The longer equilibration time required for maxadilan versus PACAP27 and the more prolonged response evoked by maxadilan suggest a distinct interaction with the PAC1 receptor.
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A 12-week contingency management intervention to promote smoking cessation in opioid-maintained individuals

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While opioid maintenance is an efficacious treatment for opioid dependence, approximately 84-94% of the population concurrently smokes cigarettes. Despite the high rates of smoking and interest in cessation, little progress has been made in developing an efficacious smoking cessation intervention for this population.

We have an ongoing NIDA-funded 12-week trial aimed at examining the efficacy of an intensive contingency management (CM) intervention for opioid-maintained individuals. During the first 2-weeks participants attend the clinic daily and can earn voucher-based reinforcement for meeting our biochemical criteria. For the remaining 10-weeks, participants are randomized to either an extended contingent (receiving vouchers contingent upon meeting our abstinence criterion) or extended noncontingent (receiving vouchers independent of smoking status) experiment group.

Thus far, 35 participants have completed the trial (33 years old, 29% male). During the first 2-weeks, participants appear to be achieving high levels of total abstinence, with 65% of samples meeting the abstinence criterion. After randomization, the contingent group appears to be maintaining superior levels of abstinence compared to the noncontingent group with 57.5% and 30% of samples meeting the abstinence criterion, respectively, $p < .05$.

Our CM intervention appears to promote high levels of smoking abstinence during the first 2-weeks, and the contingent group appears to be maintaining more smoking abstinence during weeks 3-12 than the noncontingent group. Data from the ongoing trial (N=100) will be presented.

PAC1 RECEPTOR SIGNALING WITHIN THE BED NUCLEUS OF THE STRIA TERMINALIS (BNST): IMPLICATIONS FOR ANXIETY DISORDERS

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Stressor exposure can initiate multiple neural response systems, including the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system, as well as behavioral responses often associated with fear and anxiety. The bed nucleus of the stria terminalis (BNST) has been argued to mediate all three of these responses to a sustained threat. We have previously shown that repeated variate stress increases both pituitary adenylate cyclase activating polypeptide (PACAP, *Adcyap1*) and PAC1 receptor (*Adcyap1r1*) transcript expression and immunoreactivity selectively within the oval nucleus of the BNST. Additionally, PACAP BNST infusion itself increases anxiety-like behavior. Acute BNST PACAP infusions not only increase anxiety-like behavior but also produce weight loss and decreased food intake, mimicking multiple consequences of repeated variate stress. . Importantly, inhibition of BNST PACAP signaling by continuous infusion of the receptor antagonist PACAP(6-38) into the BNST during the week of repeated variate stress can attenuate anxiety-like behavior and blunt the accompanying weight changes typically observed after stressor exposure. As PACAP binds PAC1 and VPAC receptors (VPAC1 and VPAC2) with high affinity, the selectivity of the PAC1 receptor-mediated responses in stress-induced anxiety-like behavior is unclear. *We now show that single infusions of the PAC1 receptor-selective agonist, maxadilan, into the BNST are able to produce effects similar to PACAP injection.* Single infusions of VIP, however did not produce significant weight loss. These results demonstrate that specific PAC1 receptor activation within the BNST mediates behavioral consequences of repeated stressor exposure. These data are consistent with recent associations of PACAP/PAC1 receptor dysregulation in human post-traumatic stress disorder, and these results in aggregate suggest that BNST PACAP/PAC1 expression and signaling mechanisms may be novel therapeutic targets for stress-related behavioral abnormalities.

REGULATION OF IMPULSIVE CIRCUITRY: FUNCTION OF EMOTIONAL FACES

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Impulsivity is a central clinical feature of Attention Deficit/Hyperactivity Disorder (ADHD), schizophrenia and impulse control disorders (i.e. pathological gambling, kleptomania). Cyders et al. (2007) broke impulsivity into five different factors including Positive and Negative Urgency (tendency to experience strong impulses due to positive and negative affect). As negative affect promotes problematic behaviors (Billieux et al, 2010), understanding the neurobiology of impulsivity is critical for developing behavioral interventions. The neural underpinnings of response inhibition have been well defined and involve the inferior frontal cortex and secondary motor area (Robbins 2007). However the impact of emotional content on impulsive responding is less understood. Sagaspe et al (2011) found fearful stimuli presented during a Stop Signal Task (SST) increased amygdala (emotional content) and lateral orbitofrontal cortex (response inhibition) activity, but suppressed the supplementary motor area (motor initiation). However, Albert et al. (2010) suggest that positive affect preferentially facilitates activation of inhibitory circuitry. Therefore, we hypothesized that positive and negative emotions would affect impulsive behavior through varied circuits during a SST where subjects select gender of faces expressing task-irrelevant emotions: angry, calm and happy. Behaviorally there were no significant differences in probability or speed of response inhibition related to emotion. However, differential neural activity was found during go and stop components of this task due to the presence of negative or positive emotional content, respectively. This is important because it validates the separation of urgency based on emotional type and suggests that groups varying on these factors may require differential treatment for maladaptive behaviors.

C57 MICE CHOOSE VOLUNTARY EXERCISE FOLLOWING STRESS

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A number of studies have documented the anxiolytic effects of exercise in both humans and animals. To date, there are few, if any, empirical reports of self-imposed exercise *following* a validated method of acute stress in an animal model. **PURPOSE:** To examine the effect of acute stress on voluntary wheel running in a rodent model. **METHODS:** Forty four, 8-week old male C57 mice were randomly assigned to control, stress with previous exercise, or stress with no previous exercise. Stressed mice were exposed to a series of five 0.4 mA, 0.25 sec foot shocks in a brightly lit behavioral chamber. Wheel running distances were recorded for three hours following the experimental condition. A one way ANOVA was employed to detect main effects by group, and follow up testing using Scheffe's post hoc procedure was performed for significant interactions. **RESULTS:** Results indicated a significant increase in wheel running following stress ($F=10.304$, $p<0.01$) in mice that had been exposed to prior voluntary exercise as compared to exercising controls ($p=0.012$) and stressed mice with no exercise knowledge ($p=0.021$). **CONCLUSIONS:** These results reveal an approximate ten-fold increase in voluntary wheel running distance following stress in mice with exercise experience. This would suggest that mice familiar with exercise may self-select exercise as a modality for the acute mitigation of accumulated anxiety. These results are novel, and may be the first to offer rigorous evidence of post-stress, self-selected exercise as a mechanism to reduce anxiety in an animal model. Supported by MH080935.

THE INFLUENCE OF PREGNANCY AND SYMPATHETIC INNERVATIONS ON CEREBRAL BLOOD FLOW AUTOREGULATION DURING ACUTE HYPERTENSION

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Edema formation during eclampsia occurs preferentially in the posterior brain region. One possibility is that decreased sympathetic innervations in the posterior region cause less effective cerebral blood flow autoregulation (CBFAR). Thus, we compared CBFAR between brain regions in female nonpregnant (NP) and late-pregnant (LP, E19-21) SD rats (n=8/group). Animals were anesthetized and laser Doppler probes were placed over the anterior and posterior brain regions to measure CBF simultaneously. Changes in CBF were recorded as blood pressure was increased by phenylephrine infusion. Brain water content was compared by wet:dry weights. In separate animals, we compared sympathetic innervations of posterior (PCA) to middle cerebral arteries (MCA) by immunohistochemistry of tyrosine hydroxylase (TH). In LP animals, CBFAR was less effective in the posterior region. The pressure at which CBF increased significantly was 160 in posterior vs. 170 mmHg in anterior. In both brain regions, pregnancy improved CBFAR. The pressure at which CBF significantly increased was 170 mmHg (vs. 140 in anterior) and 160 mmHg (vs. 130 in posterior). Brain water content was significantly increased in LP animals for both regions. TH nerve density was increased 2-fold in PCA vs. MCA in LP and NP rats, but pregnancy had no effect. In conclusion, diminished autoregulation in the posterior brain region during pregnancy does not appear to be related to decreased sympathetic innervations.

EVIDENCE FOR GAP JUNCTIONS IN CAJAL-RETZIUS CELLS AND INITIAL STUDIES ON THEIR SUSCEPTIBILITY TO DEVELOPMENTAL ETHANOL EXPOSURE

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Cajal-Retzius (CR) cells are among the earliest generated neurons in the cerebral cortex. They synthesize and secrete reelin, and regulate a number of developmental processes. In rodent, CR cells are prevalent during corticogenesis but their numbers decline after birth. While studies have examined the functional disposition of postnatal CR cells, relatively little has been investigated in their embryonic counterparts.

We examined CR cells in embryonic (E) and postnatal (P) Ebf2-GFP BAC transgenic mice. We noted dye-coupling between CR cells during whole-cell recording. We reasoned that, for CR cells to orchestrate cortical development over large areas of the cortex, their activity must be synchronized. Dual recording experiments revealed functional coupling between CR cells. We therefore began testing the hypothesis that CR cells express connexins during corticogenesis. E13.5 GFP+ cells were isolated and subjected to RT/PCR-based profiling for a panel of candidate connexin (Cx) transcripts. We observed the presence of mRNAs encoding Cx 32, 36, 37, 40, 43 and 45. At the level of individual CR cells, we confirmed expression for the Cx 32 and 40 transcripts. Cx 40 protein was expressed throughout the embryonic preplate, including the CR cells.

We investigated other aspects of CR cells in the developing cortex. Developmental exposure to ethanol alters neuronal migration. Since CR cells play a prominent role in neuronal migration, we asked whether exposure to a relatively low dose of ethanol affected the disposition of CR cells. We report here alterations in CR cell number and responsiveness to GABA in the postnatal cortex.

PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) IS A POTENT VASODILATOR OF MIDDLE MENINGIAL ARTERIES (MMA)

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Migraine is a debilitating neurological disorder that includes mild to severe headache. Vasodilation of MMA, located within the dura matter and innervated by the trigeminal system, may contribute to migraine headache. PACAP, a neurotransmitter present in sensory trigeminal neurons, is a vasodilator of cerebral arteries. However, the impact of PACAP on MMA is unclear. Here, we examined the ability of PACAP to dilate isolated pressurized MMA held at physiological intravascular pressure. Rat MMA (fully-dilated diameter approximately 75 microns) were dissected from the dura, cannulated on glass pipettes and placed in a 3 ml myograph chamber continuously superfused with warmed, aerated artificial cerebral spinal fluid. When pressurized to 40 mmHg, arteries developed myogenic tone representing a decrease in diameter of about 25 microns. PACAP dilated MMA in the picomolar range, with 3 pM causing dilation approximately 60% of tissue maximum. In contrast, PACAP caused significant dilation of cerebellar arteries at concentrations only above 1 nM. In summary, our data demonstrates that PACAP is approximately 1,000-fold more potent in inducing vasodilation of MMA than cerebral arteries. PACAP-induced dilation of MMA may play a role in the etiology of migraine headache. This work was supported by the Totman Trust for Medical Research and NIH (P01HL095488).

CONTEXTUAL CONTROL OF APPETITE: RENEWAL OF INHIBITED FOOD-SEEKING BEHAVIOR IN SATED RATS AFTER EXTINCTION

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A variety of evidence indicates that associative learning influences food intake. For example, the presentation of cues that have been associated with food can excite food intake in rats and humans, even when they are satiated. The current experiments extended this sort of finding by examining stimulus control of the *inhibition* of food-seeking behavior. In two experiments, rats that were fed freely on ad lib food in the home cage nevertheless learned to work for sweet or sweet/fatty food pellets by pressing a lever in a Skinner box. The initial learning occurred in a distinctive Skinner Box, Context A. Then, lever pressing was inhibited (through "extinction") by withholding the food pellets when food-seeking occurred in either Context B (a different Skinner box, Experiment 1) or in the original Context A (Experiment 2a). When extinction was complete, food-seeking was then tested in both the extinction context and in the other context (Experiment 1: Context A, Experiment 2a: Context B). In either case, inhibited food seeking returned (was "renewed") when behavior was tested outside the context in which extinction and inhibition had been learned. The results of Experiment 2, in which food seeking returned in a relatively new context (B) that had been less associated with food then the original context (A), were especially interesting. In other tests, the rats were allowed to earn pellets again. Reacquisition of food seeking was faster in the non-extinction context. The implications for eating and overeating are discussed.

ALTERED SYNAPTIC TRANSMISSION AT THE MAJOR PELVIC GANGLIA OF DIABETIC MICE

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Diabetic cystopathy (DC) is a common complication of diabetes. We have studied synaptic transmission at the major pelvic ganglia (MPG) of diabetic mice, type 1 and type 2, to determine whether impaired ganglionic neurotransmission contributes to DC. The MPG provide autonomic innervation to the urogenital organs and the distal colon. Ganglia were dissected from streptozotocin(STZ)-induced diabetic and db/db diabetic mice after 20 weeks of hyperglycemia (typically 26 weeks of age). All diabetic mice exhibited signs of DC as demonstrated previously with conscious cystometry. Membrane properties and synaptic responses were measured from MPG neurons with intracellular microelectrodes in a whole mount preparation. Resting membrane potentials were similar among the three groups (control, STZ, db/db). The input resistance of db/db neurons (101±10 Mohms), but not STZ neurons (132±13 Mohms), was significantly less than that of controls (149±15 Mohms). After-hyperpolarization (AHP) amplitudes were similar but the duration of the AHP was significantly less in db/db compared to STZ (11±0.7 ms vs. 13±0.7 ms). Also, a greater number of tonic neurons, cells which fired multiple action potentials (more than 5) with prolonged (1 sec) depolarization, were observed in ganglia of both diabetic models (control 8/17, STZ 14/18, db/db 15/20). Excitatory postsynaptic potentials (EPSPs) were evoked by focal stimulation of the pelvic nerve truck using concentric bipolar electrodes. Trains of suprathreshold stimuli (20-80 μA, 5,10 & 20 Hz, 5 sec duration) elicited similar numbers of postsynaptic action potentials in ganglia of control and diabetic animals. During stimulation, a greater number of asynchronous miniature EPSPs (mEPSPs) were observed in the diabetic ganglion cells. Following stimulation, a significantly higher number of mEPSPs were recorded in db/db mice compared to controls (at 20 Hz, 136±28 vs. 25±7). The amplitude of the mEPSPs following stimulation were also significantly reduced in the diabetic animals (4.9±0.4 mV in controls vs. 3.7±0.3 mV in STZ and 3.3±0.2 mV in db/db). The results suggest that Ca2+ dynamics regulating neurotransmitter release may be altered in diabetics and that postganglionic nicotinic function also may be compromised as previously reported. We suggest that ganglionic dysfunction occurring with diabetes could contribute to bladder dysfunction in chronically diabetic patients.

COBRE NEUROSCIENCE CELL AND MOLECULAR BIOLOGY CORE FACILITY

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The COBRE Neuroscience Cell and Molecular Biology (CMB) Core at the University of Vermont was established to serve the neuroscience community by providing the equipment and training to incorporate cell and molecular approaches into their research. The core personnel are the core director, Dr. Sheryl White, and two full time technicians: Thomm Buttolph and Edward Zelazny. The CMB core provides one of the widest ranges of molecular biology services available in academic facilities in the country, including DNA services (construct design, cloning, PCR, site-directed mutagenesis and library construction), RNA services (Quantitative PCR, RNA isolation, RT-PCR, Northerns, RNase protection and differential display analysis), protein services (SELDI-TOF mass spectrometry biomarker profiling, protein extraction, SDS-PAGE, 2D-PAGE, western blotting and gel shift assays), cell culture services (primary/cell line culturing, transfection, reporter assays, immunohistochemistry, frozen/paraffin sectioning and slide staining), as well as specialized microscopy techniques (laser capture microdissection, Neurolucida morphometrics, stereology and cell counting). The CMB core also offers training services to laboratory personnel or principal investigators wishing to learn molecular biology/cell culture technique. The equipment in the facility is available for researchers to use and includes specialized equipment such as two ABI 7500FAST systems for quantitative PCR, a SELDI-TOF mass spectrometer for proteomics and biomarker identification, a Zeiss-PALM laser microdissection system for isolation of single cells, and an Odyssey infrared imager system for Western blotting, gel shift assays and in-cell western analysis. Recent additions include a Qiagen 24 Pyrosequencer, Biotek SynergyH4 plate reader, a Countess automated cell counter, an MP Bio FASTPREP 24 cell and tissue homogenization system, and Qiagen's QIAcube fully automated spin-column based sample processor and a Qiagility liquid handling system. A full list of all the equipment and services available at the COBRE Neuroscience CMB facility can be viewed at http://www.uvm.edu/neuroscience/corecellularmolecular.html. Please visit our web site or stop by the facility in HSRF 427 and see what our core can offer for your research.

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