



# **FOURTH ANNUAL NEUROSCIENCE, BEHAVIOR AND HEALTH RESEARCH FORUM**

The University of Vermont  
Davis Center  
Livak Ballroom ~ Mansfield Room  
January 24-25, 2014

*Presented by:*



# NBH RESEARCH FORUM SCHEDULE OF EVENTS

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## FRIDAY, JANUARY 24

- 4:00pm Registration, White Pine Lounge, Davis Center 4<sup>th</sup> Floor
- 4:20pm Introductory Remarks: **George Wellman, PhD**, President, Vermont Chapter of the Society for Neuroscience, **Robert Hamill, PhD**, Professor Emeritus, Neurological Sciences, Livak Ballroom, Davis Center 4<sup>th</sup> Floor
- 4:30pm Keynote presentation: **Emanuel DiCicco-Bloom, M.D.**, Professor, Department of Neuroscience and Cell Biology, Rutgers Robert Wood Johnson Medical School, "**Autism Spectrum Disorders: Tales from the Mouse**", Livak Ballroom, Davis Center 4<sup>th</sup> Floor
- 5:30pm Reception, White Pine Lounge, Davis Center 4<sup>th</sup> Floor

## SATURDAY, JANUARY 25

- 8:15am – 8:45am ➤ Registration, Handy Room, Davis Center 4<sup>th</sup> Floor. *Light Breakfast and coffee/tea will be served.*  
➤ Poster setup, Mansfield Room, Davis Center 2<sup>nd</sup> Floor
- 8:45am Introductory Remarks: **Rae Nishi, PhD**, Director, Neuroscience, Behavior and Health Initiative, University of Vermont, Livak Ballroom, Davis Center 4<sup>th</sup> Floor

### ***Session I: Chaired by Abbie Johnson and Stephanie Spohn, Livak Ballroom, Davis Center 4<sup>th</sup> Floor***

- 9:00am **Alicia Ebert, PhD**, Assistant Professor, UVM, "**Zebrafish as a model for neurodevelopment**"
- 9:20am **Arsalan Syed**, Graduate Student, UVM, "**Differential mechanism of vasodilation by PACAP and CGRP in pressurized rat MMA**"
- 9:40am **Gene Cilento**, Graduate Student, UVM, "**The Ubiquitin Ligase Trim32 as a Regulator of Potassium Channels in the Brain**"
- 10:00am **Galen Missig**, Graduate Student, UVM, "**Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) signaling in the amygdala modulates the emotional and behavioral consequences of pain**"
- 10:20am Coffee Break, Livak Ballroom, Davis Center 4<sup>th</sup> Floor

### ***Session II: Chaired by Eric Gonzalez and Estelle Spear, Livak Ballroom, Davis Center 4<sup>th</sup> Floor***

- 10:40am **Stephanie Spohn**, Graduate Student, UVM, "**Intraluminal 5-HT<sub>4</sub> agonist treatment improves DSS and TNBS colitis in mice**"
- 11:00am **Gilbert Rahme**, Graduate Student, Dartmouth, "**A PDGF-USP1-Id2 Axis Maintains Survival of PDGF Driven Glioma Cells**"
- 11:20am **Abbie Johnson**, Graduate Student, UVM, "**Decreased Seizure Threshold during Pregnancy and Experimental Preeclampsia: Roles for GABAA Receptors and Microglial Activation**"
- 11:40am **Mary Beth Klinger-Lawrence, PhD**, Staff scientist, Med Associates/Catamount Research, "**Assessing the Time Course of Cyclophosphamide (CYP) Effects on Voiding Behavior in Female Rats**"
- 12:00pm-2:00pm Lunch and Poster Viewing/Judging, Mansfield Room, Davis Center 2<sup>nd</sup> Floor

### ***Session III: Chaired by Roman Popov and James Bishop, Livak Ballroom, Davis Center 4<sup>th</sup> Floor***

- 2:00pm **Nathan O'Connor, PhD**, Product Management/Technical Sales, MBF Bioscience, "**Managing, sharing, and analyzing terapixel images for research and education**"
- 2:20pm **Dawei Li, PhD**, Assistant Professor, UVM, "**Genetic Studies in Mental Illnesses: GWAS and Beyond**"
- 2:40pm **Hugh Garavan, PhD**, Associate Professor, UVM, "**Neuropsychosocial profiles of current – and future – adolescent alcohol misusers**"
- 3:00pm **Awards and closing remarks**

# NEUROSCIENCE, BEHAVIOR AND HEALTH RESEARCH FORUM

## POSTER SESSION

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- Poster #1     **Sensorimotor Behavioral Testing in a Mouse Model of Subarachnoid Hemorrhage**  
Evelyn A. Bulkeley, Masayo Koide, George C. Wellman
- Poster #2     **The effects of voluntary exercise or methylphenidate on learning a set-shift task during development**  
Meghan C. Eddy, Katherine J. Stansfield, & John T. Green
- Poster #3     **Cerebellar Secretin Modulates Acquisition and Extinction of Eyeblink Conditioning**  
Jason R. Fuchs, Gain M. Robinson<sup>1</sup>, Anthony D. Morielli, John T. Green
- Poster #4     **Infusion of pituitary adenylate cyclase-activating polypeptide (PACAP) into the bed nucleus of the stria terminalis (BNST) produces a stress response in female rats**  
Gick, N., Edwards, S., Choi, I., Roelke, E., Lezak, K.R., Toufexis, D., Braas, K.M., May, V., Hammack, S.E.
- Poster #5     **Effects of Chronic vs Cycling estrogen treatment on acquisition, retention and expression of spatial memory**  
Olga Lipatova & Donna Toufexis
- Poster #6     **Effects of Acute Nicotine Administration on Emotional Impulsivity**  
Geoffrey J. Schaubhut, Janina K. Bowen, Emily C. Mazzulla, Sarahjane L. Dube, Alexandra S. Potter
- Poster #7     **Differential Effects of Nicotine and Ritalin on Working Memory in ADHD**  
Eli Sepkowitz, Geoffrey Schaubhut, Sarahjane Dube and Alexandra Potter
- Poster #8     **Contextual control of instrumental actions vs. habits**  
Eric A. Thrailkill & Mark E. Bouton
- Poster #9     **Data-driven dynamic mapping of the brain**  
Nicholas A. Allgaier, Hugh Garavan, Josh C. Bongard, and Christopher M. Danforth
- Poster #10    **Applying Artificial Neural Networks to fMRI Data**  
Aaron Morton, Hugh Garavan, Robert Snapp
- Poster #11    **MRI T2 Measurements Post Febrile Status Epilepticus Predict Performance On An Active Avoidance Task**  
J.M. Barry, M. Choy, C. Dube, P.P. Lenk-Santini, R.C. Scott, T.Z. Baram, G.L. Holmes
- Poster #12    **Can establishing a regular meditation practice reduce college students' physiological responses to stressful testing situations and impact their social cognition**  
Kahn, H., Raghunath, R., Calhoun, C., Lesenskyj, A., Weinert-Stein, M., Cronise, K., & Sellers, J.
- Poster #13    **AZD3480, a Novel Nicotinic Receptor Agonist, for the Treatment of Attention-Deficit/Hyperactivity Disorder in Adults**  
Alexandra S. Potter, Geoffrey Dunbar, Emily Mazzulla, David Hosford, Paul A. Newhouse

- Poster #14 **Getting rid of resurgence: Implications for contingency management treatments**  
Sydney Trask, Scott T. Schepers, & Mark E. Bouton
- Poster #15 **The Role of Human Histidyl-tRNA Synthetase Mutations in Human Diseases, Type IIIB Usher Syndrome and Peripheral Neuropathy**  
Jamie Abbott, Bin Deng, Ying Wai Lam, Christopher Francklyn, and Susan Robey-Bond
- Poster #16 **Identification of protein networks disrupted by a mutation in HARS**  
Susan Robey-Bond, Julia Fields, Ying-Wai Lam and Christopher Francklyn
- Poster #17 **Molecular Characterization of Plexins: Signaling Mechanisms and Developmental Expression**  
Rachael Bassett and Bryan Ballif
- Poster #18 **Endothelial and Smooth Muscle Derived Neuropilin Like Protein is Required for Proper Development of the Retina in *Danio rerio***  
Ryan M. Joy, Bryan A. Ballif Ph.D., Alicia M. Ebert Ph.D.
- Poster #19 **A role for FGF8a in neurovasculature signaling in developing zebrafish**  
Erin Wysolmerski, Kathyana Santiago-Mangual, Alicia Ebert
- Poster #20 **Functional role for transforming growth factor-beta (TGF- $\beta$ ) signaling following cyclophosphamide (CYP)-induced cystitis in female rats**  
Eric J Gonzalez, Margaret A Vizzard
- Poster #21 **TRPV4 blockade reduces urinary bladder dysfunction by increasing bladder capacity and decreasing micturition frequency following RVS in male rats**  
Liana Merrill & Margaret Vizzard
- Poster #22 **Increased voiding frequency is associated with oxidative stress and ATP production in cyclophosphamide (CYP)-induced cystitis in rats**  
A. Peterson, S. Malley, B. Girard, M. Kosofsky, D. Lambert, M.A. Vizzard
- Poster #23 **Measuring Kv1.2 potassium ion channel trafficking in real time**  
Amy Duncan-Smith and Anthony Morielli
- Poster #24 **PAC1 receptor internalization is required for activation of the MEK/ERK intracellular signaling cascade in HEK 293 cells stably expressing the PAC1 receptor**  
Victor May, Thomas Buttolph, Beatrice A. Girard, Todd Clason, Laura A. Merriam and Rodney L. Parsons
- Poster #25 **Targeting the  $\alpha 5$  nicotinic acetylcholine receptor (nAChR) subunit as a treatment for neuroblastoma**  
Vanessa Ochoa, Loen Hansford, David Kaplan, and Rae Nishi
- Poster #26 **L-amino acid taste: Are multiple receptors involved?**  
Shreoshi Pal Choudhuri, Rona J. Delay, and Eugene R. Delay
- Poster #27 **Increased asynchronous neurotransmitter release at parasympathetic major pelvic ganglion neurons in diabetic mice: Implication for altered Ca<sup>2+</sup> homeostasis**  
John D. Tompkins, Rodney L. Parsons

- Poster #28 **Fingolimod Promotes Primary Sensory Afferent Growth in Developing Chicken Embryo**  
Michelle McNamara and Cynthia Forehand
- Poster #29 **A PLC $\gamma$ 1-dependent, Force-sensitive Signaling Network in the Myogenic Constriction of Cerebral Arteries**  
Albert L. Gonzales, Ying Yang, Michelle N. Sullivan, Lindsey Sanders, David C. Hill-Eubanks, Mark T. Nelson, and Scott Earley
- Poster #30 **Inhibition of nitric oxide synthase restores cerebral artery tone in a rodent model of traumatic brain injury**  
Nuria Villalba, Tram L. Tran, Mark T. Nelson, George C. Wellman and Kalev Freeman
- Poster #31 **Subarachnoid hemorrhage suppresses KV1 and KV2 currents via different mechanisms in rat parenchymal arteriolar myocytes**  
Koide M, O'Connor KP, Pappas AP, Syed AU and Wellman GC
- Poster #32 **ROS-dependent and -independent MMP activation leads to KV current suppression in cerebral artery myocytes after SAH**  
Masayo Koide and George C. Wellman
- Poster #33 **Contribution of Rho Kinase to TRPM4-Mediated Myogenic Tone in Cerebral Parenchymal Arterioles**  
Yao Li, Rachael L. Baylie, Matthew J. Tavares, Joseph E. Brayden
- Poster #34 **Impairment of Neurovascular Coupling by Chronic Stress**  
Thomas Longden, Fabrice Dabertrand, Sayamwong E Hammack, Mark T Nelson
- Poster #35 **The increased amplitude of spontaneous Ca $^{2+}$  events in astrocytic endfeet parallels the inversion of neurovascular coupling after subarachnoid hemorrhage**  
Anthony C. Pappas, Masayo Koide, George C. Wellman
- Poster #36 **TRPV4 Ca $^{2+}$  sparklets in myoendothelial projections (MEPs) regulate vascular function**  
Swapnil K. Sonkusare, Adrian D. Bonev, Thomas Dalsgaard, Luis F. Santana, Michael I. Kotlikoff, Mark T. Nelson
- Poster #37 **Activation of ATP-Sensitive Potassium (KATP) Channels underlies vasodilation to PACAP, but not CGRP, in Pressurized Rat Middle Meningeal Artery**  
Arsalan U. Syed, Masayo Koide, Victor May, George C. Wellman
- Poster #38 **A Decade of the Journal IMPULSE: Growth and Impact**  
Mansky, B., Georgakas, J., Casler, A., Kreuzman, D., Spollen, K., Cronise, K., Jones LS
- Poster #39 **COBRE NEUROSCIENCE CELL AND MOLECULAR BIOLOGY CORE FACILITY**  
Sheryl White, Cindy Forehand and Rodney Parsons
- Poster #40 **Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) in the Amygdala: Origin and Coexpression**  
May V, Missig G, Braas KM, Vizzard MA, Hammack SE
- Poster #41 **The Ubiquitin Ligase Trim32 as a Regulator of Potassium Channels in the Brain**  
Gene Cilento and Anthony Morielli

## PLATFORM TALK ABSTRACTS

*Listed by order of presentation*

### **Differential mechanism of vasodilation by PACAP and CGRP in pressurized rat MMA**

Arsalan U. Syed<sup>1</sup>, Masayo Koide<sup>1</sup>, Victor May<sup>1,2</sup>, George C. Wellman<sup>1</sup>

Departments of Pharmacology<sup>1</sup> and Neurological Sciences<sup>2</sup>, University of Vermont College of Medicine, Burlington, VT

Migraine is a complex neurological disorder that often presents as an intense unilateral headache accompanied by nausea, photophobia and other neurological symptoms. Activation of the trigeminovascular system and/or the sphenopalatine ganglia involving the release of the neuropeptides pituitary adenylate cyclase activating polypeptide (PACAP) and calcitonin gene related peptide (CGRP) has been implicated in vasodilation of the middle meningeal artery (MMA) and the sensation of migraine headache. However, the mechanism by which these two peptides exert their vasodilatory effect on the MMA is unclear. Activation of distinct receptors for PACAP and CGRP have been linked to activation of adenylyl cyclase in vascular smooth muscle. In addition, CGRP receptors have also been identified in vascular endothelial cells. Activation of cyclic AMP-dependent protein kinase has been shown to induce vasodilation via multiple mechanisms including phosphorylation and activation of smooth muscle  $K_{ATP}$  channels in a variety of vascular beds. In the present study our goal is to determine the role of  $K_{ATP}$  channels in vasodilation mediated via PACAP and CGRP in rat MMA. In isolated, pressurized MMAs both PACAP and CGRP induced significant vasodilation, although PACAP ( $EC_{50} \sim 1$  pM) exhibited  $\sim 1,000$ -fold greater potency compared to CGRP ( $EC_{50} \sim 1$  nM). PACAP-induced MMA dilation was completely abolished by the  $K_{ATP}$  channel inhibitor, glibenclamide ( $10 \mu M$ ). In marked contrast, glibenclamide did not influence MMA dilation caused by CGRP. Further, N-Nitro-L-Arginine (L-NNA), a nitric oxide synthase inhibitor, had no effect on dilation caused by PACAP or CGRP. These observations demonstrate that PACAP dilates MMA via activation of vascular  $K_{ATP}$  channels, while CGRP acts through an alternative pathway. Thus, it appears that PACAP and CGRP contribute to the etiology of migraine via two distinct mechanisms. Therapeutic approaches targeting a combination of both PACAP and CGRP may be more effective than targeting either of these peptides alone in alleviating migraine headache. This work was supported by the Totman Medical Research Trust Fund, the Peter Martin Brain Aneurysm Endowment and the NIH (P01 HL095488, P30 RR032135 and P30 GM103498).

## Platform Talk Abstract

### The Ubiquitin Ligase Trim32 as a Regulator of Potassium Channels in the Brain

Gene Cilento, Neuroscience Graduate Student, Department of Pharmacology/Neurological Sciences

Dynamic regulation of the voltage gated potassium channel Kv1.2 strongly influences neuronal excitability. Ubiquitylation, particularly monoubiquitylation, is one means of signaling such regulation by tagging ion channels for nondegradative, endocytosis. We used mass spectrometry (MS) to identify ubiquitylation sites within Kv1.2 purified from the brain. Additionally, MS analysis of Kv1.2 interacting proteins identified the ubiquitin ligase Trim32. *In vitro*, Trim32 was able to ubiquitylate Kv1.2 directly, supporting Trim32 as the source of Kv1.2 ubiquitylation in the brain. In cultured cells, Trim32 modulates Kv1.2 surface trafficking through mechanisms that either involve or are independent of ubiquitylation, depending on growth conditions. Additionally, overexpression of Trim32 alters the phosphorylation state of Kv1.2, proposing a complex model of Kv1.2 modulation that likely involves cross-talk between post-translational modifications. Altogether, our study demonstrates a new mechanism for the regulation of Kv channels in the brain and provides new insight towards neuronal excitability control.

## **Platform Talk Abstract**

### **Pituitary adenylate cyclase activating polypeptide (PACAP) signaling in the amygdala modulates the emotional and behavioral consequences of pain**

Galen Missig, Carolyn W. Roman, Margaret A. Vizzard, Karen M. Braas, Sayamwong Hammack, and Victor May

Department of Neurological Sciences, University of Vermont College of Medicine, Burlington, VT 05405

Chronic pain is frequently associated with depression, sleep dysregulation, anxiety abnormalities, panic disorder and post traumatic stress disorder (PTSD). The high comorbidity between chronic pain and stress-related disorders may suggest a common underlying vulnerability pathway. In the central nervous system, pituitary adenylate cyclase activating polypeptide (PACAP) signaling is demonstrated to play a role in stress, pain, and other emotion-related processes. PACAP-expressing fibers are abundant in the lateral capsular division of the central amygdala (CeLC of CeA), a key site of integration for sensory and limbic pathways, and recent evidence suggests that CeLC PACAP may be predominantly projection terminals from distant nuclei. Through immunocytochemical colocalization, anatomical tracing, and lesion studies, we show that PACAP-containing fibers in the CeLC originate from neurons in the lateral parabrachial nucleus (PBN) as part of the spino-parabrachioamygdaloid tract carrying nociceptive signals to the limbic system. As this pathway has been evidenced to mediate emotional aspects of pain, we next examined the functional role of CeA PACAP signaling in stress and pain-related behaviors. Similar to our previous work in the bed nucleus of the stria terminalis (BNST), PACAP infusion into the CeA was anxiogenic, resulting in anxiety-like behaviors on the elevated plus maze. Unlike the BNST, CeA PACAP signaling produced small changes in weight gain suggesting that anxiety-like and stress-related feeding behaviors can be dissociated. In a second set of experiments examining nociceptive related responses, CeA PACAP infusion resulted in a robust thermal hypersensitivity with more modest changes in mechanical sensitivity. In aggregate, these results suggest that PACAP signaling in the amygdala via the pontine parabrachioamygdaloid circuit may be one of the central mechanisms modulating the emotional and behavioral consequences of pain.



## **Platform Talk Abstract**

### **Intraluminal 5-HT<sub>4</sub> agonist treatment improves DSS and TNBS colitis in mice**

Stephanie N. Spohn, Brigitte Lavoie, Sarah J. MacEachern, Jane A. Roberts, Rebecca L. Wilcox, Keith A. Sharkey and Gary M. Mawe

Department of Neurological Sciences, University of Vermont, Burlington, VT and Department of Physiology and Pharmacology, University of Calgary, Calgary, AB, Canada

Mucosal application of 5-HT<sub>4</sub> agonists in the colon increases enterocyte Cl<sup>-</sup> secretion, goblet cell degranulation, and enterochromaffin cell release of 5-HT. As these actions have protective features, we tested the hypothesis that stimulation of epithelial 5-HT<sub>4</sub> receptors would reduce inflammation in mice with experimental colitis. Colitis was induced in mice using 4% DSS in the drinking water or by colonic enema containing 2.5% TNBS in 50% ethanol. Mice were treated daily via enema with the 5-HT<sub>4</sub> agonist, tegaserod, or with vehicle for 5-7 days. Disease activity index (DAI) was evaluated based on weight, stool consistency, and fecal blood; macroscopic damage scores (MDS) were determined by colon length and thickness, and presence of ulcers. Barrier function was evaluated in vivo by intracolonic administration of Evans Blue, and ex vivo by measuring transcolonic fluorescein (FITC) flux across in an Ussing chamber. Treatment with tegaserod improved the DAI and MDS, and normalized the histological appearance of most colons as compared to DSS-vehicle controls. In DSS-inflamed animals treated with tegaserod, less Evans Blue penetrated the epithelium into the outer layers of the colon, as compared to vehicle treated inflamed animals. Furthermore, in colons from DSS-inflamed animals, acute application of tegaserod to the mucosal chamber reduced the flux of FITC to the serosal chamber. Taken together, these data suggest that activation of epithelial 5-HT<sub>4</sub> receptors reduces inflammation and improves barrier function. These findings support the hypothesis that luminal administration of 5-HT<sub>4</sub> agonists could have a protective effect in intestinal inflammation. Supported by DK62267.

## Platform Talk Abstract

### A PDGF-USP1-Id2 Axis Maintains Survival of PDGF Driven Glioma Cells

Gilbert J. Rahme<sup>1,3</sup>, Alison L. Young<sup>1,2,3</sup>, Yasuyuki Hitoshi<sup>2,3</sup>, Mark A. Israel<sup>1,2,3</sup>

<sup>1</sup>Department of Genetics

<sup>2</sup>Department of Pediatrics

<sup>3</sup>Norris Cotton Cancer Center

Geisel School of Medicine at Dartmouth, Dartmouth College, NH, USA

GJR, PhD Graduate Student

ALY, Laboratory Technician

YH, Previous Post-Doctoral Associate

MAI, Professor of Genetics and Pediatrics, Director of the Norris Cotton Cancer Center

Glioblastoma multiforme is the most aggressive form of brain tumor that typically leads to mortality even after current therapy. Specifically, proneural GBM, a subgroup of glioblastoma characterized by gain-of function alterations in PDGFR signaling, is particularly resistant to current therapy. Although the importance of PDGF signaling in GBM is well understood, it is unclear what signaling pathways downstream PDGF drive pathogenesis. To study PDGF signaling in GBM, we utilized a transgenic mouse model developed in our laboratory in which PDGF-B expression in GFAP-positive cells is under the control of a Tet-off system and can be regulated by doxycycline. In the absence of doxycycline, these animals invariably develop high-grade glioma and GBM driven by PDGF. Inhibitor of DNA binding (Id) genes are frequently overexpressed in tumors of different tissue origins including glioma and are thought to be oncogenic. Here we report that Id2 is regulated post-translationally by PDGF signaling and is essential for the survival of PDGF-driven glioma cells. Further, genetic deletion of Id2 induced apoptosis and delayed mortality due to tumorigenesis in mice overexpressing PDGF. We found that USP1, a ubiquitin specific peptidase, is upregulated by PDGF signaling to stabilize Id2 protein levels. Our data describe a signaling cascade downstream PDGF that is required for survival of GBM cells and suggest that inhibition of Id2 could serve as a therapeutic strategy against GBM with alterations in PDGF signaling.

## Platform Talk Abstract

### Decreased Seizure Threshold during Pregnancy and Experimental Preeclampsia: Roles for GABAA Receptors and Microglial Activation

Abbie C. Johnson<sup>1</sup>, Sarah M. Tremble<sup>2</sup> and Marilyn J. Cipolla<sup>2,3,4</sup>

<sup>1</sup>Neuroscience Graduate Program; Depts. of <sup>2</sup>Neurological Sciences, <sup>3</sup>Pharmacology, and <sup>4</sup>Obstetrics, Gynecology & Reproductive Sciences, University of Vermont, Burlington, VT

#### Background:

Preeclampsia (PE) is a leading cause of maternal morbidity and mortality worldwide. De novo seizure can occur during PE, but also during seemingly uncomplicated pregnancies, suggesting pregnancy may increase risk for seizure independent of PE. We hypothesized pregnancy is a state of increased seizure susceptibility, and further increased in PE. We determined the effect of pregnancy and PE on seizure threshold and investigated mechanisms by which pregnancy and PE may affect seizure threshold including neuroinflammation and GABAA receptor expression.

#### Methods:

Seizure threshold was determined by measuring the amount (mg/kg) of pentylenetetrazol required to elicit seizure. Rats that were nonpregnant (NP, n=7), late-pregnant (LP, n=6) or with reduced uteroplacental perfusion pressure on a high cholesterol diet (RUPP+HC, n=6) to model PE, were compared. In the cerebral cortex protein expression of the GABAAR  $\delta$  subunit was determined (n=3/group) and microglial activation assessed morphologically (NP, n=6; LP, n=8; RUPP+HC, n=3).

#### Results:

Seizure threshold for NP, LP and RUPP+HC rats was  $65 \pm 15$  vs.  $37 \pm 10$  mg/kg ( $p < 0.01$ ) and  $12 \pm 4$  mg/kg ( $p < 0.01$ ), respectively. Decreased seizure threshold in pregnancy was associated with reduced protein expression of the GABAAR  $\delta$  subunit from  $0.97 \pm 0.03$  AU in NP to  $0.56 \pm 0.06$  AU in LP ( $p < 0.01$ ). Pregnancy did not cause microglial activation as the % of activated cells was similar to NP ( $12 \pm 2$  vs.  $9 \pm 3$  %;  $p > 0.05$ ), however, RUPP+HC rats had significant microglial activation ( $35 \pm 2$  %;  $p < 0.05$ ).

#### Conclusion:

These results suggest the maternal brain is hyperexcitable during pregnancy that is further augmented in PE. Pregnancy appears to lower seizure threshold through decreased GABAAR subunit expression whereas neuroinflammation likely increases the risk of seizure during PE. Understanding how pregnancy and PE affect seizure susceptibility may allow for better prevention of eclampsia.

## **Platform Talk Abstract**

### **Assessing the Time Course of Cyclophosphamide (CYP) Effects on Voiding Behavior in Female Rats**

Mary Beth Klinger-Lawrence

Med Associates/Catamount Research

Cyclophosphamide-induced cystitis is widely used in rodents as a pre-clinical model for Painful Bladder Syndrome/Interstitial Cystitis (PBS/IC). PBS/IC is a significant and unresolved clinical problem, with an estimated 3.3 million adult women affected in the United States. Symptoms vary widely between patients, but generally include urinary frequency, urgency, and pain in the bladder and pelvic area. While CYP is commonly used and well-established as a model for this disorder, a search of the literature shows numerous variations in study parameters involving CYP, including dosage, method of bladder function analysis, and timing of functional studies post-dose.

We aim to define the functional progression of CYP-induced cystitis after a single dose of CYP (150 mg/kg) up to 72 hours post-injection using metabolic cages. Software was used to record data from analytical balances located beneath each metabolic cage during 24 hour analysis. Animals were analyzed prior to treatment (baseline) and again during three different time periods post-CYP injection: 0-24 hours post-injection, 24-48 hours post-injection, and 48-72 hours post-injection. The total 24 hour number of voids was significantly increased as compared to vehicle controls in the 0-24 hour and 48-72 hour groups ( $p \leq 0.05$ ). Average inter-micturition interval (IMI), total void volume, and average individual void volumes were quantified over 24 hour period.

With this work we hope to illuminate a clear time course for effects of CYP on bladder function using non-invasive metabolic cage testing, and in turn define an ideal window for the testing of potentially therapeutic compounds.

## **Platform Talk Abstract**

### **Genetic Studies in Mental Illnesses: GWAS and Beyond**

Dawei Li

Department of Microbiology and Molecular Genetics

Department of Computer Sciences

Neuroscience, Behavior and Health Initiative

Almost all mental illnesses are heritable. Genetic association studies have successfully identified mental illness-associated genes. However, for each individual disease those identified genes are limited in numbers, and all these genes cumulatively explain a small proportion of the known or estimated inherited risk, and the rest genetic risk is unknown (missing heritability). In this presentation, I will take our current Illumina SNP genotyping array-based genetic study of addiction as an example to introduce the under-studied aspects that may help identify new disease genes, including rare variant, copy number variation, and gene-environment interaction.

## **Platform Talk Abstract**

### **Neuropsychosocial profiles of current – and future – adolescent alcohol misusers**

Hugh Garavan

Department of Psychiatry, University of Vermont

A comprehensive account of the causes of alcohol misuse must accommodate individual differences in biology, psychology and environment, and must disentangle cause and effect. Animal models can demonstrate the effects of neurotoxic substances; however, they provide limited insight into the psycho-social and higher cognitive factors involved in the initiation of substance use and progression to misuse. One can search for pre-existing risk factors by testing for endophenotypic biomarkers in non-using relatives; however, these relatives may have personality or neural resilience factors that protect them from developing dependence. A longitudinal study has potential to identify predictors of adolescent substance misuse, particularly if it can incorporate a wide range of potential causal factors, both proximal and distal, and their influence on numerous social, psychological and biological mechanisms. Here, we apply machine learning to a wide range of data from a large sample (n=692) of adolescents to generate models of current and future adolescent alcohol misuse that incorporate brain structure and function, individual personality and cognitive differences, environmental factors (including prenatal cigarette and alcohol exposure), life experiences, and candidate genes. These models were accurate and generalized to novel data, and point to life experiences, neurobiological differences and personality as important antecedents of binge drinking. By identifying the vulnerability factors underlying individual differences in alcohol misuse, these models shed light on the etiology of alcohol misuse and suggest targets for prevention.

# POSTER ABSTRACTS

*Listed by Poster Number*

## **Poster #1**

### **Sensorimotor Behavioral Testing in a Mouse Model of Subarachnoid Hemorrhage**

Evelyn A. Bulkeley, Masayo Koide, George C. Wellman

Department of Pharmacology, University of Vermont College of Medicine, Burlington, VT

Subarachnoid Hemorrhage (SAH) is an often lethal form of stroke, for which few medical interventions exist to counteract long-term sensorimotor deficits in survivors. Functional animal models of stroke can provide valuable information about the extent of sensorimotor deficits as well as insight into the biological cause of the deficits and the potential for rehabilitation. Here, six sensorimotor behavioral tests were conducted on SAH (endovascular perforation model), sham-operated and un-operated (control) mice. An assessment score was used to record the results of the animals 0, 1, 2, 3, and 4 days post-surgery. Our results demonstrate a statistically significant difference in the performance of SAH mice compared to control mice ( $p < 0.01$  Day 1, 2, 3 and  $p < 0.05$  Day 4), as well as a statistically significant difference between SAH model and Sham-operated mice day ( $p < 0.01$  Day 1, 2, 3, and  $p < 0.05$  Day 4). The greatest difference in the performance between SAH animals and the sham-operated and control groups were detected by the tail suspension test, pole test, and wire hang test. This study demonstrates sensorimotor deficits occur in mice with SAH induced via the endovascular perforation model. Further, we show that a battery of relatively simple behavioral tests can be used to assess SAH-induced neurological deficits in SAH model mice.

**The effects of voluntary exercise or methylphenidate on learning a set-shift task during development**

Meghan C. Eddy, Katherine J. Stansfield, & John T. Green

Department of Psychology, University of Vermont

Exercise-related improvements in cognitive control and working memory have been shown in children (Buck et al., 2008; Hillman et al., 2009) and are of particular interest in the context of ADHD, as current psychostimulant treatments, such as methylphenidate (MPH) carry with them several concerns. There is some indication that exercise during development may ameliorate ADHD symptoms, though data suggest that exercise does not affect adults and adolescents identically (Hopkins et al., 2011). We have previously shown that two weeks of exercise in adult rats improves Set 1 performance of a set-shift task (Eddy et al., 2011). Considering these findings, the purpose of these experiments was to explore the effects of exercise or MPH during development on set-shift performance. Set-shifting is a test of cognitive flexibility requiring a discrimination between rewarded and unrewarded arms in a T maze. Set 1 (initial discrimination) relies upon the dorsolateral striatum (DLS) and Set 2 (“shift”), the dorsomedial striatum (DMS) and medial prefrontal cortex (mPFC). Adolescent rats that exercised for two weeks or received two weeks of daily MPH showed improvement on Set 2. When rats were treated as adolescents and treatment stopped for two weeks before testing (i.e., tested as adults) there was only an improvement in Set 2 performance in animals receiving MPH. These data suggest that exercise or MPH during development can improve this DMS/mPFC mediated learning, and only MPH effects persist into adulthood if treatment is discontinued. Future experiments will look at dopamine and norepinephrine transporters in the DLS and mPFC, respectively.



**Cerebellar Secretin Modulates Acquisition and Extinction of Eyeblink Conditioning**

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Eyeblink conditioning (EBC) relies on interactions between Purkinje cells (PCs) in cerebellar cortex and neurons in the interpositus nucleus (IPN) of the deep cerebellar nuclei. Basket cells exert powerful control over PC output and thus, exert powerful control over acquisition and extinction of eyeblink CRs. BC axon terminals express a high concentration of the  $\alpha$ -subunit of the voltage-gated K<sup>+</sup> channel, Kv1.2 and regulation of this channel has been linked to the neuropeptide secretin. Our previous research has shown that intra-cerebellar infusions of either a Kv1.2 blocker or secretin enhance EBC. In the current research, we examined the effects of intra-cerebellar infusions of secretin or a secretin antagonist on acquisition and extinction of EBC. In Experiment 1, rats were infused with either the secretin receptor antagonist, 5-27 secretin, or vehicle immediately prior to the first 3 days of EBC. Rats that received 5-27 secretin showed slower learning than the vehicle-treated rats. In Experiments 2 and 3, rats received either secretin or the 5-27 secretin prior extinction to examine their effects on extinction of EBC. Rats that received secretin prior to extinction were impaired during this learning. Our working model is that secretin released by PCs modulates EBC by reducing surface levels of Kv1.2 at BC terminals, thereby increasing inhibition of PCs.

## Poster #4

### **Infusion of pituitary adenylate cyclase-activating polypeptide (PACAP) into the bed nucleus of the stria terminalis (BNST) produces a stress response in female rats**

Gick, N., Edwards, S., Choi, I., Roelke, E., Lezak, K.R., Toufexis, D., Braas, K.M., May, V., Hammack, S.E.

Recent gene association studies have implicated pituitary adenylate cyclase-activating peptide (PACAP) systems in several psychiatric disorders associated with stressor exposure, and we have implicated the bed nucleus of the stria terminalis (BNST) as a critical brain region for the regulation of stress-related behaviors by PACAP in male rats. However, we have also reported that PACAP dysregulation is associated with post-traumatic stress disorder (PTSD) in women, and estradiol (E2) treatment upregulates PACAP and PAC1 receptor transcript in the BNST of female rats. Hence, in the present study we assessed the effects of intra-BNST PACAP on stress responding in ovariectomized female rats with or without E2 replacement. In female rats, BNST PACAP produced anorexia and weight loss, and led to an increase in plasma corticosterone, mimicking the response to an acute stressor. The effects of BNST PACAP did not interact with E2 treatment. These results suggest that BNST PACAP activation may be important for the central regulation of stress responding in female rats, and support a growing literature implicating PACAP in stress and anxiety.

**Effects of Chronic vs Cycling estrogen treatment on acquisition, retention and expression of spatial memory**

Olga Lipatova & Donna Toufexis

Estrogen has been shown to either enhance or impair memory in female rats. The type of experimental paradigm or treatment regiment used to test the effect of estrogen on learning is one of the contributing factors for the disparate findings. In order to assess the effect of different estradiol (E2) treatments on several elements of cognition, we trained ovariectomized (OVX) E2-replaced female rats in an open-field tower maze task (OFTM) designed to test spatial memory in a relatively stress-free manner. Previously, we showed that during hippocampal-based place-learning in the OFTM, chronic E2 replacement in OVX female rats, maintained using slow-release E2 pellets, did not affect acquisition of spatial memory, enhanced spatial memory retention, and disrupted expression of memory following a switch in the start position. In the present experiment, we duplicated the effect of chronic E2-pellet replacement, using daily E2 sc injections. In addition, we included a cyclic regiment with E2 injections administered once every 4 days at proestrus levels. In the present experiment we also included rats trained with a striatum-based response learning in addition to the hippocampus-based place-learning procedure. Results showed that cyclic E2 replacement facilitated the acquisition of spatial memory during place learning, but neither E2 regiment affected acquisition of response learning. Cue retrieval via daily sc injections and handling throughout the retention period prevented previously observed impairment in performance following the 21-day retention interval. In addition, both regiments of E2 disrupted the expression of spatial memory when rats were tested with a novel start location in the OFTM. Interestingly, this E2-mediated disruption was specific to the rats using place learning. These results suggest that E2 regiment differentially affects the acquisition of spatial learning that is mediated by the hippocampus, and that hippocampal-based learning may be more sensitive to disruption following environmental changes.

## Poster #6

### Effects of Acute Nicotine Administration on Emotional Impulsivity

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Attention Deficity/Hyperactivity Disorder (ADHD) is associated with earlier onset, higher levels, and frequent relapses of cigarette smoking versus controls. These differences are linked to impulsive behaviors, which are reduced following acute nicotine self-administration. Although impulsivity and nicotine craving are related to negative affect, how they interact to produce behavioral disturbances is unknown. This study examined the effects of acute nicotine on emotional impulsivity (i.e. the inability to regulate behavior following emotional stimulation) in non-smokers with and without ADHD. We hypothesized that ADHD subjects would show greater sensitivity to emotional stimuli and reduced activation of inhibitory circuitry, which would reverse during nicotine exposure.

Twelve (6 ADHD, 6 CTRL) young adults (age 18–25) completed two study days including fMRI scanning while performing the Faces Stop Signal Task. Before scanning, transdermal nicotine or placebo was administered in a double blind design.

Results indicated a difference in the processing of inhibitory and emotional stimuli dependent on ADHD profile. Specifically, ADHD versus CTRL had reduced activity in the frontal cortex during inhibition in the PLC condition, not seen during the NIC condition. Emotion caused increased parietal activation during inhibition of negative compared to positive stimuli across subjects, but was maximal in ADHD subjects during the PLC condition.

This demonstrates that nicotine can regulate activity in neural circuitry related to the integration of emotional information during response inhibition. Therefore, emotional impulsivity may be a factor for nicotine use in ADHD subjects, but future research is needed to elaborate the relationship between nicotine, emotion and impulsivity.

**Differential Effects of Nicotine and Ritalin on Working Memory in ADHD**

Eli Sepkowitz, Geoffrey Schaubhut, Sarahjane Dube and Alexandra Potter  
University of Vermont, Department of Psychiatry

Attention-Deficit/Hyperactivity Disorder (ADHD) is associated with a wide range of neuropsychological deficits, including deficits in working memory. While Ritalin, the hallmark ADHD drug, reduces symptoms and improves some aspects of cognitive functioning, there is little association between the clinical and cognitive response. The well-documented relationship between cigarette smoking and ADHD, and the known positive effects of nicotine on cognitive function, has prompted investigation into treatments of ADHD via stimulation of nicotinic acetylcholine receptors (nAChRs). Clinically, understanding the effects of different pharmacotherapies on specific cognitive operations could help to identify the cognitive mechanism for specific symptoms and may guide individualized treatment in the future. Thus in this study we sought to understand the differential effects of manipulating nAChR function versus stimulating dopaminergic and norepinephrine receptors (the presumed mechanism of action for Ritalin) on working memory.

22 non-smoking young adults (11 healthy controls and 11 with ADHD-Combined subtype) received acute nicotine (NIC), mecamylamine (MEC), methylphenidate (Ritalin; RIT) and placebo (PLC) on separate days. The verbal N-Back task was used to assess working memory.

There was significant speeding of hit reaction time (RT) observed in the ADHD group following NIC administration on the 2- and 3-back conditions, as well as a trend for speeding associated with RIT on the 2-back condition. In the control group, there was a significant slowing of RT following MEC administration on the 3-back condition. Additionally, MEC interfered with response accuracy as working memory load increased. These findings suggest the importance of the nAChR system in working memory.

**Contextual control of instrumental actions vs. habits**

Eric A. Thrailkill & Mark E. Bouton

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With limited amounts of training, instrumental behavior is controlled by the motivational status of its goal (i.e., it is therefore considered a goal-directed action). However, with extended training, actions are thought to become habits that are more insensitive to the motivational status of the goal. Amount of training, as well as whether the behavior has been reinforced on ratio or interval schedules of reinforcement, has been thought to contribute to the development of actions vs. habits. Recently, our lab has shown that instrumental behavior can be highly sensitive to changes in context; that is, instrumental responding weakens when it is tested in a new situation. Here, we investigated whether this sensitivity to context change depends on the behavior's putative status as an action or a habit. Experiment 1 replicated the context-switch effect in groups of rats receiving extensive or limited training to perform instrumental lever pressing on either ratio and yoked-interval schedules. Experiments 2a and 2b replicated the context-switch effect in groups that received even more limited training. Reinforcer devaluation tests suggested that the behavior was controlled by both goal-directed and habit processes; the context switch appeared to weaken the habit, but not the action, component. Experiment 3 extended these findings by showing that an extensively-trained response, which was shown to be completely insensitive to reinforcer devaluation, still remained sensitive to a context switch. The results suggest that the context controls instrumental behavior over a very wide range of training conditions, but mainly supports habitual rather than goal-directed behavior processes.

**Data-driven dynamic mapping of the brain**

Nicholas A. Allgaier<sup>1,2,3</sup>, Hugh Garavan<sup>4,5</sup>, Josh C. Bongard<sup>2,3,6</sup>, and Christopher M. Danforth<sup>1,2,3</sup>

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A detailed characterization of the human brain, its structural and functional underpinnings, remains on the frontier of modern science. Neurological research is important not only for its intrinsic interest, but for the purpose of better understanding (diagnosing and treating) neurological disorder as well. Happily, along with many other fields, neuroscience is entering an era of "Big Data" in which a new approach is possible: allow the data to suggest a theory, which may then be validated against independent data. In this poster we summarize ongoing work applying this approach to the analysis of functional Magnetic Resonance Imaging (fMRI) data from a 243-subject study of the adolescent, resting-state brain. Computational techniques inspired by biological evolution are employed to discover and mathematically characterize interactions among regions of interest (ROI), without making linear or univariate assumptions. Statistics of the resulting interaction relationships comport with recent independent work, constituting a preliminary validation. Moreover, new nonlinear interactions are suggested that are not discoverable by current methods of analysis. Upon further successful testing, the methodology will be employed to enhance the theoretical framework within which we understand and model the human brain.

**Applying Artificial Neural Networks to fMRI Data**

Aaron Morton, Hugh Garavan, Robert Snapp

NERVE Lab & College of Engineering and Mathematical Sciences at UVM

An artificial neural network is a computational model used to classify patterns in large, complex datasets. The strengths of these networks make them particularly well suited to the classification of fMRI and phenotypic data; here a subset of the IMAGEN dataset is considered. IMAGEN is a multi-site, longitudinal study of adolescent neurodevelopment with a specific interest in understanding the development of adolescent drug and alcohol use. It contains genetic, neuroimaging and behavioral measures (personality, cognition, mental health etc) on a sample of 2,400 fourteen year olds. Using data collected at age 14, including 6 brain activation measures obtained using fMRI, neural networks were trained and evaluated. These networks attempted to predict whether or not subjects would become binge drinkers in the future, using data collected at age 16 to verify accuracy. The best networks had an AUC of .74 and an average accuracy of 70%. These initial results are promising in that they establish that the network was able to successfully detect patterns within the data with which it could successfully predict future binge drinking. Future testing with different feature sets and more varied network architectures will be investigated to optimize the prediction accuracy.



## **MRI T2 Measurements Post Febrile Status Epilepticus Predict Performance On An Active Avoidance Task**

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Febrile seizures are the most common type of seizure seen in young children. Mounting evidence from both animal models and patient data suggest that Febrile Status Epilepticus (FSE) may have long-term deleterious consequences and put some at risk of diminished cognitive capacity. Identifying those individuals at risk for cognitive impairment and discovering the mechanisms responsible would provide opportunities for therapeutic intervention. We now show that MRI T2 measures are predictive of performance on an active avoidance spatial task. T2 levels are lower than normo-thermic controls in whole brain as well as all regions of interest approximately 120 minutes post FSE. However, T2 levels from FSE animals unable to learn the avoidance task are significantly higher than FSE animals that are able to learn. While T2 levels in the whole brain were predictive of spatial cognitive ability, T2 in the hippocampus and the basolateral amygdala were also good predictors. Lesion studies suggest that these two brain structures are necessary to learn and perform the active avoidance task suggesting that increased T2 levels may correlate with compromised function of these brain regions. Place cells recorded in the hippocampus tended to be of similar quality for control and FSE animals that were able to learn the task while FSE non-learners had significantly larger firing fields and a significantly higher in-field firing rate. This suggests that excitatory pyramidal cells are less inhibited in the FSE non-learners than controls or FSE learners. Inter-ictal spikes were found in the LFPs of most FSE animals. There was no difference in the rate of inter-ictal spikes in FSE learners or non-learners. This suggests that the presence of inter-ictal spikes did not affect learning of the active avoidance task.

**Can establishing a regular meditation practice reduce college students' physiological responses to stressful testing situations and impact their social cognition**

Kahn, H.<sup>1</sup>, Raghunath, R.<sup>2</sup>, Calhoun, C.<sup>3</sup>, Lesenskyj, A.<sup>1</sup>, Weinert-Stein, M.<sup>1</sup>, Cronise, K.<sup>1&2</sup>, & Sellers, J.<sup>3</sup>  
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Meditation positively affects the emotional and cognitive responses of adults. Few studies have examined meditation practices with adolescents or young adults. This pilot study explores the impact of loving kindness meditation on college-aged adults. Two classes were tested. As part of their course, one class underwent a weekly 15 min meditation practice for 14 weeks. The semester progressed normally for the control class. After 9 weeks, students completed surveys to assess stress (Perceived Stress Scale), social adjustment (Work and Social Adjustment Scale), narcissism (Narcissistic Personality Inventory [NPI-40]), and mindfulness (5 Facet Mindfulness Questionnaire). There were no stress or social adjustment differences between classes. As compared to all other participants, meditating males showed higher awareness and nonjudgmental scores on the 5 Facet Mindfulness Questionnaire. Also, meditators had higher self-sufficiency scores on the NPI-40. After 14 weeks, stress responses were measured while students completed a subset of the graduate record examination. Blood pressure (BP), electrodermal responses (EDR), cortisol responses and anxiety self-reports were collected. BP was measured at baseline, before testing, 15 minutes into testing and post-test. Systolic BP did not differ between groups, but diastolic BP rose for all participants during testing. EDR's were measured for the first 10 minutes of testing and no differences between groups were seen. Post-test, students completed the State Trait Anxiety measure and provided salivary cortisol samples. Those data are being analyzed. This study is a preliminary investigation to determine if a regular meditation practice can attenuate the academic and social pressures of college aged adults.

Middlebury College Senior Research Fellowship, Middlebury College, Middlebury, VT 05753

**AZD3480, a Novel Nicotinic Receptor Agonist, for the Treatment of Attention-Deficit/Hyperactivity Disorder in Adults**

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**Background:** Laboratory studies have found that acute stimulation of nicotinic acetylcholine receptors improves cognition in adult ADHD. Clinical trials of nicotinic agonists have been mixed, underscoring the need to understand the mechanisms for individual differences in clinical response. Using cognitive models within a clinical trial framework may provide insight into these differences.

**Methods:** This was a within-subjects, randomized, placebo controlled double-blind trial of the nicotinic agonist AZD3480 (also termed TC-1734) at doses of 5 mg, 50 mg and placebo in adults with ADHD. The order of the two week treatment periods was randomized and a three week wash out separated each drug treatment period. Response inhibition (Stop Signal Task; SST) and clinical efficacy (Investigator Rated Conners Adult ADHD Rating Scale; CAARS-INV) were the apriori primary outcome measures of cognitive and clinical effects. We hypothesized that AZD3480 treatment would improve SST performance and clinical symptoms (CAARS-INV Total ADHD Symptoms Score).

**Results:** 30 subjects were randomized, with 24 included in the intent to treat analyses. SST performance and Total ADHD symptoms were significantly improved with 50 mg of AZD3480. CAARS-INV ratings of inattention, memory problems, and emotional lability/impulsivity were significantly improved with 50 mg of AZD3480.

**Conclusions:** These results support previous work suggesting that nicotinic agonists are viable as treatments for adult ADHD. Measuring cognitive endophenotypes related to both the disorder and mechanism of the treatment, may help further rational drug development for dimensional features that cross cut psychiatric disorders.

**Getting rid of resurgence: Implications for contingency management treatments**

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In resurgence, an extinguished instrumental response (R1) recovers when a behavior meant to replace it (R2) is also extinguished. The resurgence effect is similar to relapse that has been shown after “contingency management” (CM) treatments used to reduce unhealthy behavior in humans (e.g., smoking, drug-taking, or overeating). In CM, like resurgence experiments, an unwanted behavior is reduced by reinforcing an alternate behavior. However, unlike resurgence experiments, an abstinence contingency is also imposed; reinforcement is not delivered unless the client has abstained from the unwanted behavior. Two experiments with rats examined the effect of adding an abstinence contingency to the resurgence paradigm and then investigated its underlying mechanism. In Experiment 1, adding an abstinence contingency reduced (but did not eliminate) resurgence. However, a control group that earned reinforcers at the same time points without an abstinence requirement showed the same effect, suggesting that the abstinence requirement reduced resurgence merely by making reinforcers less frequent. Experiment 2 tested this hypothesis in a standard resurgence design by manipulating the rate at which reinforcers could be earned in Phase 2. Rats that received rich reinforcement schedules during response elimination showed resurgence, whereas rats that received leaner schedules did not. Theoretically, the leaner schedules allowed the animal to learn that R1 was extinguished in the context of infrequent reinforcement, a context more like that of resurgence testing. The data are thus consistent with other research from our laboratory suggesting that relapse can be reduced by encouraging generalization from the response elimination context to new contexts.

**The Role of Human Histidyl-tRNA Synthetase Mutations in Human Diseases, Type IIIB Usher Syndrome and Peripheral Neuropathy**

Jamie Abbott, Bin Deng, Ying Wai Lam, Christopher Francklyn, and Susan Robey-Bond

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Histidyl-tRNA synthetase (HARS) is a Class II aminoacyl-tRNA synthetase that catalyzes the attachment of histidine to its cognate tRNA (tRNA<sup>His</sup>) during protein synthesis. Recently, a mutation in hHARS has been linked to Type IIIB Usher syndrome in a small cohort of Amish pediatric patients, and is the first example linking cytoplasmic hHARS to a sensorineural disease. Type IIIB Usher syndrome presents clinically as deaf-blindness in the first decades of life. The hHARS mutation encodes a Y454S substitution that is localized to the surface of the anticodon-binding domain - catalytic domain interface. Of note, another group has described an hHARS mutation in a patient suffering from peripheral neuropathy that encodes a substitution R137Q. This substitution alters what is predicted to be an important dimer stabilizing interaction, and R137Q hHARS is dominant lethal in yeast. When R137Q hHARS is expressed in *C.elegans*, the animals had aberrant commissural axonal processes and locomotor defects. We have established an expression system for purification of the hHARS enzyme, and begun biochemical characterization of the WT and Y454S hHARS with *in vitro* transcribed human tRNA<sup>His</sup>. Both the WT and Y454S mutant hHARS enzymes exhibit considerable aminoacylation activity in initial studies, suggesting that the Y454S substitution does not compromise enzyme activity. Additionally, proteomic approaches are being employed to identify post-translational modifications of WT and Y454S hHARS in an attempt to identify signaling pathways associated with hHARS. Through these approaches, we seek to understand the biochemical reasoning behind this substitution and how it results in hearing loss.

**Identification of protein networks disrupted by a mutation in HARS**

Susan Robey-Bond, Julia Fields<sup>2</sup>, Ying-Wai Lam<sup>2</sup> and Christopher Francklyn

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A syndrome comprising deafness, blindness, and fever-induced hallucinations was recently discovered in Amish children. Designated Usher syndrome Type IIIB, it is caused by a mutation in an enzyme that has not previously been associated with Usher syndrome, histidyl-tRNA synthetase (HARS). HARS is a class II aminoacyl-tRNA synthetase required for protein production in cells, and no mutation in this enzyme has previously been linked to human disease. That children with this HARS mutation are born healthy, and develop the syndrome in early childhood, suggests that HARS may have a second, previously unknown, function. Recently, non-canonical roles have been discovered in other members of the class II AARS family. Any secondary functions of HARS would likely be mediated through binding to other proteins. We determined the interactions of HARS and mutant HARS with other cellular proteins, specifically in cells derived from embryonic mouse inner ears. A co-immunoprecipitation, coupled with mass spectrometry, has been performed using either neuronal (N33) or epithelial (mechanosensory precursor) cells (E36), comparing the proteins captured by HARS or Y454S. With respect to functional annotation of the hits obtained by this approach, proteins involved in transcription, translation, organelle trafficking and cytoskeleton, and signal transduction are highly represented. Several Rab proteins (monomer G proteins of the Ras superfamily), which are involved in vesicle trafficking were identified preferentially immunoprecipitated by HARS, but not Y454S. Proteins identified from E36 cells are mostly related to aminoacylation activity, and include sets of proteins both overlapping and distinct from N33 cells.

## Molecular Characterization of Plexins: Signaling Mechanisms and Developmental Expression

Rachael Bassett and Bryan Ballif

Plexins are essential for proper neuronal migration during vertebrate development. Plexin A1 and Plexin A2 are transmembrane receptors that have been shown to transduce primarily repulsive signals from Semaphorins leading to growth cone collapse in migratory neurons.<sup>1</sup> Very little is known about the molecular mechanisms of these two pathways. Evidence of tyrosine phosphorylation of the cytoplasmic region of the Plexins has been shown and tyrosine kinases have been implicated in Plexin signaling, including the Src family kinase, Fyn.<sup>2</sup> Fyn activation is thought to be needed to phosphorylate proteins downstream responsible for inducing the growth cone collapse response.<sup>3</sup> How the binding of Semaphorins activates Fyn is still unknown. In order to understand the role Fyn might be playing in Plexin signaling, we investigated its role in phosphorylating Plexins at a highly conserved intracellular tyrosine phosphorylation site. The high conservation between vertebrate Plexin A1 and Plexin A2 orthologs made it plausible to propose similar hypotheses about both of the receptors: we hypothesize (1) that Fyn induces phosphorylation of Plexin A1 at Y1606 and of Plexin A2 at Y1605, and (2) that Fyn binds to the Plexin receptors upon the phosphorylation of the proposed sites. To determine the importance of this phosphorylation and the relative contribution of Fyn, we present here the establishment and characterization of a cell-based assay, as well as an *in vivo* model (*Danio rerio*).

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## Poster #18

### **Endothelial and Smooth Muscle Derived Neuropilin Like Protein is Required for Proper Development of the Retina in *Danio rerio***

Ryan M. Joy, Bryan A. Ballif Ph.D., Alicia M. Ebert Ph.D.

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Endothelial and smooth muscle derived neuropilin like protein (ESDN) is a transmembrane receptor that is implicated in the programming of neuronal stem cells during embryonic development due to its tyrosine-dependent interaction with the Crk family adaptor proteins (CrkL) which play a critical role in neuronal migration. ESDN is localized to neuronal tissues including the retina, therefore, we hypothesized that ESDN was required for proper development of the retina. We therefore conducted a study in zebrafish to determine the role of ESDN in eye development. Knockdown of ESDN resulted in incomplete development of the retina and reduced numbers of retinal ganglion cells (RGCs). To assess phenotypes, we scored brain innervation by the RGCs in whole-mount embryos in addition to RGC cell counts in sectioned embryos during the first three days of development. The preliminary data supports ESDN's involvement in neuronal development, specifically in RGCs. Further work will consist of more detailed elucidation of the phenotype, rescuing using tyrosine residue mutant constructs, and identification of the ligand. Our preliminary data suggest ESDN is required for proper development of the RGCs in zebrafish.



**A role for FGF8a in neurovasculature signaling in developing zebrafish**

Erin Wysolmerski, Kathyana Santiago-Mangual, Alicia Ebert  
Department of Biology, University of Vermont

Fibroblast growth factors (FGFs) are critical in many aspects of embryonic development and other cellular functions including apoptosis, cell adhesion, and proliferation. We identified mRNA expression of FGF8a in the retinal ganglion cells (RGCs) and its receptor FGFR1 in surrounding retinal vasculature of 2 day-old zebrafish. Antisense morpholino knockdown of FGF8a resulted in a significant reduction in the number of RGCs and also a reduction in the corresponding tectal innervation. In addition, FGF8a morphant embryos have mispatterned retinal vasculature, suggesting a role in neurovascular signaling. It has previously been reported that zebrafish survive and develop normally for 7 days without blood flow as it receives nutrients by simple diffusion. To rule out hypoxia, we utilized the silent heart mutant, which lacks cardiac troponin t resulting in embryos without blood flow, as heart contractility does not initiate. Cell counts from these fish have however, shown a loss in RGC numbers. Therefore, using immunohistochemistry, we looked to see if loss of RGCs was due to lack of proliferating cells using pHH3 or increased cell death using active caspase 3 in both silent heart and FGF8a mutant fish. We hypothesize that the reduced cell numbers will be due to a lack of proliferating cells and not cell death. To further our understanding of this intricate developmental system we intend to look closer into the connection between the RGCs and the developing vasculature.

**Functional role for transforming growth factor-beta (TGF- $\beta$ ) signaling following cyclophosphamide (CYP)-induced cystitis in female rats**

Eric J Gonzalez, Margaret A Vizzard  
Department of Neurological Sciences

Our previous studies demonstrated that TGF- $\beta$  and its cognate receptors significantly alter their transcriptional and translational profiles following CYP-induced cystitis. However, the functional role of intracellular TGF- $\beta$  signaling in urinary bladder dysfunction remains unknown. The goal of these studies was to determine the role of TGF- $\beta$  in urinary bladder function using a rat model of CYP-induced cystitis (48 hours; 150 mg/kg; i.p.) and a TGF- $\beta$  type I receptor antagonist (SB505124, 5  $\mu$ M). Intravesical infusion of SB505124 (5  $\mu$ M) was performed and bladder function determined using conscious, freely moving rats with an open outlet. Consistent with previous studies, CYP-treated animals exhibited significantly ( $p \leq 0.01$ ) increased voiding frequency and decreased bladder capacity, void volume and intercontraction intervals. TGF- $\beta$  type I receptor blockade in CYP-treated rats significantly ( $p \leq 0.01$ ) decreased voiding frequency and increased bladder capacity (2.5-fold), void volume (2.5-fold) and intercontraction intervals (2.5-fold) relative to baseline. TGF- $\beta$  type I receptor blockade did not affect micturition pressures (filling, threshold, peak) in CYP-treated rats. These results suggest: (1) a functional role for TGF- $\beta$  signaling in the afferent limb of the micturition reflex following CYP-induced cystitis and (2) targeting TGF- $\beta$  type I receptors at the level of the urinary bladder may be an effective strategy for reducing voiding frequency with urinary bladder dysfunction.

E. Gonzalez, M.A. Vizzard. Functional role for transforming growth factor-beta (TGF- $\beta$ ) signaling following cyclophosphamide (CYP)-induced cystitis in female rats. Program No. 657.11. 2013 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2013. Online.

**TRPV4 blockade reduces urinary bladder dysfunction by increasing bladder capacity and decreasing micturition frequency following RVS in male rats**

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Stress exacerbates symptoms of functional lower urinary tract disorders including interstitial cystitis (IC)/bladder pain syndrome (BPS) and overactive bladder (OAB) in humans, but mechanisms contributing to symptom worsening are unknown. Multiple transient receptor potential (TRP) channels expressed in the urinary bladder may act as sensors of stretch and/or chemical irritation and are thought to play functional roles in OAB and IC/BPS. Previously, we have shown that repeated variate stress (RVS) significantly increases voiding frequency and decreases bladder capacity and void volumes, and increases somatic sensitivity, in rats. In order to examine the role of TRPV4 in bladder function following RVS, stressed rats were exposed to a 7-day RVS paradigm with a single stressor presented daily. Bladder function was evaluated with open outlet, conscious cystometry, followed by intravesical administration of a TRPV4 antagonist HC067047 (1  $\mu$ M) or agonist GSK1016790A (3  $\mu$ M) for 30 min. Administration of the antagonist significantly ( $p \leq 0.01$ ) increased bladder capacity (2.3-fold) and void volume and decreased voiding frequency (2.4-fold) in rats that were exposed to RVS. Administration of the agonist significantly ( $p \leq 0.01$ ) increased voiding frequency (3.7-fold) and decreased void volumes (4.2-fold) and bladder capacity (3.7-fold) in control rats compared to the pre-drug state. In addition, there were no significant differences in voiding frequency, bladder capacity, and void volumes between controls rats that received agonist administration and rats that had been exposed to RVS. These results, in combination with previous data, imply a functional role of TRPV4 in urinary bladder function.

**Increased voiding frequency is associated with oxidative stress and ATP production in cyclophosphamide (CYP)-induced cystitis in rats**

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IC/BPS is a chronic pain condition characterized by pressure, discomfort, and pain related to the urinary bladder. Although the etiology of IC/BPS is unknown, the majority of biopsies from BPS/IC patients reveal inflammation. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated by inflammation result in oxidative stress and contribute to urinary bladder dysfunction. Using a rat model of urinary bladder inflammation induced by CYP, we determined: (1) the expression of oxidative stress markers (3-nitrotyrosine (3-NT), ROS/RNS, superoxide anion) in the bladder and (2) the functional role of ROS/RNS in urinary bladder dysfunction with a superoxide scavenger and superoxide dismutase mimetic, Tempol (1 mmol/L in drinking water) combined with conscious cystometry. In CYP-treated (4 hr; 150 mg/kg, i.p.) rats, 3-NT, a biomarker of oxidative protein damage, significantly ( $p \leq 0.01$ ) increased in urinary bladder. The dichlorofluorescein (DCF) assay, a measure of ROS/RNS, was significantly ( $p \leq 0.01$ ) reduced in urinary bladder with 4 hr CYP treatment. Dihydroethidium (DHE) staining, a marker of superoxide production, significantly ( $p \leq 0.01$ ) increased in detrusor smooth muscle with 4 hr CYP treatment. CYP-treatment (4 hr) increased ATP content in urine and increased purinergic receptors (P2X2 and P2X3) expression in urinary bladder. In CYP-treated (4 hr) rats, Tempol increased bladder capacity, reduced voiding frequency and decreased peak micturition pressure compared to CYP-treated rats without Tempol. Tempol dramatically reduced ATP production in urine samples from CYP-treated rats. We demonstrate that: (1) CYP-induced cystitis is associated with an oxidative stress state in the bladder; (2) CYP-induced cystitis increased ATP content in urine and purinergic receptors in bladder; (3) Tempol treatment improves bladder function and decreases ATP content in urine.

**Measuring Kv1.2 potassium ion channel trafficking in real time**

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Kv1.2 is a voltage gated potassium channel involved in the modulation of neuronal excitability. Kv1.2 function is controlled by endocytic trafficking, a dynamic process involving the movement of Kv1.2 from the cell surface into endocytic vesicles and back to the cell surface via a recycling pathway. Most studies examining ion channel trafficking in neurons utilize single end-point measurements after a regulatory stimulus. While informative, this approach does not readily resolve the temporal dynamics of channel trafficking and therefore overlooks a key dimension of ion channel regulatory effects on neuronal function. The goal of this experiment was to determine the feasibility of monitoring endocytic trafficking of Kv1.2 in real time using pHluorin, a pH sensitive green fluorescence protein. To accomplish this, a pHluorin-Kv1.2 fusion protein in which pHluorin was inserted into the first extracellular loop of Kv1.2 and the pH insensitive fluorescent protein mCherry was fused to the channel's N-terminus, was generated. When endocytosed, the extracellular portion of Kv1.2 becomes exposed to the acidic lumen of the endocytic vesicle, reducing pHluorin's fluorescence. The fluorescence of mCherry, however, is unaffected. In conjunction with live cell imaging, the ratio of pHluorin to mCherry fluorescence can thereby be used as a real-time measure of Kv1.2 trafficking between the cell surface and endosomes. Drugs previously shown to evoke changes in Kv1.2 trafficking were perfused into a medium containing cells, and then imaged using a fluorescence microscope. Forskolin was expected to inhibit the endocytosis of Kv1.2, while both carbachol and serum were expected to increase endocytosis.

**PAC1 receptor internalization is required for activation of the MEK/ERK intracellular signaling cascade in HEK 293 cells stably expressing the PAC1 receptor**

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Prior studies indicate that internalization of the PACAP/PAC1 complex and formation of a signaling endosome mediates multiple cellular functions following PAC1 receptor activation (May et al, J Biol Chem 285: 9749, 2010; Merriam et al., J Neurosci: 33: 4614, 2013). We tested in the present studies, using transfected HEK 293 cells stably expressing the GFP-tagged PAC1 receptor, whether PACAP activation of the MEK/ERK kinase signaling cascade was affected by environmental or pharmacological interventions that blunted PAC1 receptor internalization. Fluorescent imaging documented a PACAP-induced internalization of the PAC1 receptor, an effect suppressed at room temperature (~25°C) or by treatment with the small molecule clathrin inhibitor Pitstop 2 or the dynamin I/II inhibitor dynasore. None of these treatments inhibited PACAP-induced increase in HEK PAC1 receptor cell cAMP production. In contrast, the PACAP-stimulated ERK phosphorylation, determined by Western Blot analysis, was significantly decreased under the pharmacological and temperature treatment conditions that suppressed PAC1 receptor endocytosis. Activation of adenylyl cyclase by forskolin, increased cAMP levels comparable to that seen with PACAP, but forskolin treatment did not recapitulate the PACAP-induced activation of ERK phosphorylation. Fura-2 measurements indicated 25 nM PACAP consistently initiated transient increases in intracellular calcium at both room temperature (22-25.5°C) and when the cells were kept at warmer temperatures (35-37°F). These results suggest that the PAC1 receptor-stimulation of adenylyl cyclase and transient elevation of intracellular Ca<sup>2+</sup> is mediated at the plasma membrane, whereas in contrast, the PACAP-induced activation of the MEK/ERK kinase pathway requires PACAP/PAC1 receptor internalization. Supported by NIH grant NCRR P30RR032135/NIGMS P30 GM103498.

**Targeting the  $\alpha 5$  nicotinic acetylcholine receptor (nAChR) subunit as a treatment for neuroblastoma**

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Neuroblastoma is a pediatric extra-cranial solid tumor that accounts for only 7% of malignancies in children less than 15 years of age, but makes up 15% of pediatric oncology deaths despite surgery, chemotherapy, radiation and bone marrow transplant. Thus it is an aggressive cancer for which new treatments are badly needed. Neuroblastoma occurs in sympathoadrenal progenitor cells, and tumors are formed in the adrenal medulla and sympathetic ganglia. In an effort to identify a drug that targets neuroblastoma, we identified, MG 624, a nicotinic acetylcholine receptor (nAChR) antagonist, by testing its efficacy in killing that tumor initiating cells (TICs) isolated from the bone marrow of patients with stage IV neuroblastoma (unpublished data). We discovered that several different TIC cell lines, several neuroblastoma cell lines (SKN-SY5Y; SH-KCN, SH-KCNR, and SH-EP), and many primary neuroblastoma tumors showed highly elevated levels of transcripts encoding CHRNA5 ( $\alpha 5$  nAChR subunit) when compared to normal human sympathetic ganglia. There was no detectable expression of the  $\square$ HRNA3 ( $\alpha 3$ ) and CHRNB4 ( $\beta 4$ ) and no relative difference in CHRNA7 ( $\alpha 7$ ) and CHRNB2 ( $\beta 2$ ) nAChR transcripts. In contrast, skin-derived neural precursors did not express detectable levels of any nAChR subunit transcript. We hypothesize that the  $\alpha 5$  subunit contributes to the neuroblastoma phenotype, and forms a novel functional nAChR with subunits  $\alpha 7$  and  $\beta 2$ , and that this receptor subtype is the target of the drug MG 624. We have confirmed that MG624 also kills SH-EP cells with the same concentration dependence as on neuroblastoma TICs. We are presently testing whether SH-RNAs targeted to CHRNA5 on SH-EP cells prevents MG624- mediated killing.

**L-amino acid taste: Are multiple receptors involved?**

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Research with L-glutamate (glu), a prototypical L-amino acid that activates umami taste pathways, suggests two G-protein coupled receptors, T1R1+T1R3 and t-mGluR4, are important in umami taste. Glu along with other L-amino acids and nucleotides such as inosine monophosphate (IMP) act as natural flavor enhancers. The taste enhancement property of Glu is further potentiated by the presence of IMP. HEK cell expression data showed that IMP potentiated the response for other L-amino acids as well (Nelson et al., 2002). However, very little is known about the peripheral taste mechanisms for detection and transduction of L- amino acids other than Glu. Using calcium imaging of isolated TSCs and taste buds of mice we asked if: (1) TSCs respond to different L-amino acids and show synergy in the presence of IMP, (2) receptors other than T1R1/T1R3 are involved in the detection of L-amino acids, In our calcium imaging study, we found that L-amino acids elicit a variety of response patterns among TSCs. For example, a single TSC may respond to more than one L-amino acid, but not all L-amino acids elicit a response in the same TSC. Further, TSCs also show synergy for different L-amino acids when mixed with IMP. We also found that TSCs from T1R3<sup>-/-</sup> mice respond to different L-amino acids and show response potentiation in the presence of IMP. These findings suggest the involvement of multiple taste receptors for detecting L-amino acids.



**Increased asynchronous neurotransmitter release at parasympathetic major pelvic ganglion neurons in diabetic mice: Implication for altered  $\text{Ca}^{2+}$  homeostasis**

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Bladder and erectile dysfunction are common urologic complications of diabetes. Diabetic dysautonomia is thought to contribute to both conditions. To determine whether disruption of ganglionic neurotransmission contributes to a loss of pelvic organ function with diabetes, we investigated synaptic transmission at parasympathetic, major pelvic ganglion (MPG) neurons in control and diabetic mice. In contrast to what has been reported for superior cervical ganglion neurons, cholinergically mediated excitatory postsynaptic potentials (EPSPs) in diabetic mice were suprathreshold ( $>98\%$ ) for action potential generation. Tetanic stimulation (5, 10, 20 Hz) elicited asynchronous neurotransmitter release, observed as miniature EPSPs (mEPSPs) during and after stimulation, permitting a quantitative assessment of postganglionic cholinergic receptor sensitivity. mEPSP amplitudes (recorded at  $-60$  mV) were reduced slightly in STZ (type 1 diabetic) mice, but unaffected in db/db (type 2 diabetic) mice. Intriguingly, the number of post-tetanic mEPSPs was substantially greater in db/db mice. Greater post-tetanic mEPSP frequency was observed in two models of type 2 diabetes (db/db and ob/ob) at 6, 12 and 26 wks of age. The frequency of mEPSPs was increased by increasing  $[\text{Ca}^{2+}]_o$  and/or depolarizing mitochondrial membrane potentials. The results indicate, depression of ganglionic nicotinic receptor function with diabetes does not reduce ganglionic neurotransmission at parasympathetic MPG neurons in diabetic mice; yet, impaired  $\text{Ca}^{2+}$  homeostasis with type 2 diabetes, potentially associated with altered mitochondrial function, disrupts presynaptic regulation of neurotransmitter release. Ongoing experiments seek to determine whether altered nerve terminal  $\text{Ca}^{2+}$ -homeostasis contributes to neurodegeneration with type 2 diabetes.

## **Fingolimod Promotes Primary Sensory Afferent Growth in Developing Chicken Embryo**

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Fingolimod, a Sphingosine-1 phosphate (S1P1) agonist is a recently approved oral treatment for relapsing forms of multiple sclerosis. The primary therapeutic mechanism of Fingolimod is to bind to S1P1 receptors on the surface of lymphocytes. Receptor internalization then prevents lymphocytes from leaving lymphoid tissue and infiltrating the central nervous system thereby stifling neurodegeneration (Gasperinin et al., 2012). In addition to its effects on immune cells, Fingolimod is also known to increase brain derived neurotrophic factor (BDNF) secretion in neurons (Deogracias et al., 2012). BDNF is a neurotrophic factor known to promote neuron survival and neurite outgrowth in embryonic, primary sensory afferents (Lindsay et al., 1985). During neural development, dorsal root ganglia (DRG) cells produce a peripheral and central process. The central process of DRG cells extends into the spinal cord at the dorsal root entry zone. These processes branch along the rostral-caudal axis in the white matter until they invest within the grey matter of the cord. Our laboratory is interested in the regulation of longitudinal growth of sensory axons in the spinal cord. Previous research in our laboratory demonstrates that inhibition of BDNF with a function blocking antibody results in a significant attenuation of DRG longitudinal axon extension.

The current experiments are designed to test the following hypotheses: 1) enhancing BDNF secretion promotes DRG axon outgrowth within the longitudinal pathway and 2) the S1P1 receptor contributes to axonal growth in the longitudinal pathway. To test these hypotheses embryonic day 5 (E5) chicken embryos were micro-injected with DiI in an in vitro preparation of ganglia and spinal cord, which was then cultured for 5 hours in the presence of Fingolimod or artificial cerebral spinal fluid (ACSF) with 1% DMSO vehicle. Fingolimod application enhances BDNF release and accelerated axon growth at concentrations greater than 50ng/ml. The result is significantly longer axons than control treatments ( $P < 0.01$ ). Secondly, we applied S1P1 agonists, SEW2817 and antagonists, W123 in which we found a significant increase and decrease in axon extension respectfully. Our data suggest 1) in vivo, Fingolimod treatment enhances BDNF release and axon outgrowth and 2) axon outgrowth involves S1P1 receptor activity. These results indicate a role for Fingolimod in modulating axon outgrowth, which may contribute to recovery from axonal damage in multiple sclerosis.

## **A PLC $\gamma$ 1-dependent, Force-sensitive Signaling Network in the Myogenic Constriction of Cerebral Arteries**

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Maintaining constant blood flow in the face of fluctuations in blood pressure is a critical autoregulatory feature of cerebral arteries. An elevation of pressure within the artery lumen causes the vessel to constrict through depolarization and contraction of the encircling smooth muscle cells (SMCs). This pressure-sensing mechanism involves the activation of two types of transient receptor potential (TRP) channels: TRPC6 and TRPM4. However, despite the fundamental importance of this mechanism, the signaling network linking pressure and membrane depolarization remains obscure. Here, we provide evidence for the convergence of two pressure-sensing pathways—a convergence that hinges on the specific activation of the  $\gamma$ 1 isoform of phospholipase C (PLC $\gamma$ 1). Inositol 1,4,5-trisphosphate (IP3) generated by PLC $\gamma$ 1 in response to pressure sensitizes IP3 receptors (IP3Rs) to Ca<sup>2+</sup> influx mediated by the mechanosensitive TRPC6 channel, synergistically elevating IP3R-mediated Ca<sup>2+</sup> release to activate TRPM4 currents, leading to smooth muscle depolarization and vessel constriction. Using proximity ligation assays, we further demonstrate co-localization of PLC $\gamma$ 1 and TRPC6 with TRPM4, supporting the concept that this functional linkage reflects the operation of a force-sensitive, local signaling network comprising PLC $\gamma$ 1, TRPC6, TRPM4 and IP3Rs. We also show that Src tyrosine kinase activity is necessary for stretch-induced TRPM4 activation and myogenic constriction, consistent with the known activation of PLC $\gamma$  isoforms by Src. We conclude that contraction of cerebral artery SMCs requires the integration of direct and indirect pressure-sensing signaling pathways and their convergence on IP3Rs, which mediate localized Ca<sup>2+</sup>-dependent depolarization through the activation of TRPM4.

**Inhibition of nitric oxide synthase restores cerebral artery tone in a rodent model of traumatic brain injury**

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Cerebral autoregulation is altered after traumatic brain injury (TBI) reflecting an impairment in myogenic tone that may contribute to patient morbidity. We hypothesized that TBI alters nitric oxide (NO)-dependent signaling mechanisms leading to decreased cerebral vascular tone. We studied isolated cerebral arteries from adult rats after moderate fluid percussion TBI or sham surgery. We found that both endothelial and smooth muscle (SM) NO levels—indexed by 4,5-diaminofluorescein (DAF-2) fluorescence—were increased in cerebral arteries from TBI animals. Arteries from TBI animals exhibited decreased cytosolic SM  $\text{Ca}^{2+}$  and reduced myogenic tone compared to controls. Endothelial removal restored myogenic constriction in TBI animals. Further, inhibition of NO synthase with N $\omega$ -L-arginine (L-NNA) restored both myogenic tone and SM  $\text{Ca}^{2+}$ , and reduced DAF-2 fluorescence in arteries from TBI animals. The guanylyl cyclase inhibitor (ODQ) and protein kinase G (PKG) inhibitor (RP-8-Br-cGMPS) both elicited enhanced constrictions in TBI animals. Further, constriction caused by inhibition of SM large-conductance  $\text{Ca}^{2+}$ -activated potassium (BK) channels with paxilline was augmented in TBI arteries. Addition of 30 nM clamped NO to control arteries provided vasodilation equivalent to that observed in TBI and increased the DAF-2 signal to a comparable level observed in untreated TBI preparations. These data demonstrate that trauma causes persistent changes in endothelial NO production underlying profound cerebral artery dilation, and provides a quantitative measure of the degree of NO elevation, which is on the order of 30 nM.

**Subarachnoid hemorrhage suppresses KV1 and KV2 currents via different mechanisms in rat parenchymal arteriolar myocytes**

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Subarachnoid hemorrhage (SAH) leads to membrane potential depolarization in arteriolar myocytes and enhanced arteriolar tone in brain parenchymal arterioles (Nystoriak et al, 2011). However the mechanism underlying this augmented constriction is currently unknown. Here, we studied the impact of SAH on voltage-gated potassium (KV) currents. In rat parenchymal arterioles from control animals, KV1.2, 1.5, 2.1 and 2.2 mRNA was expressed, and KV1 (correolide-sensitive) and KV2 (stromatoxin-sensitive) currents were detected by whole cell K<sup>+</sup> current measurement. Both KV1 and KV2 currents as well as total KV (4-AP-sensitive) currents were significantly suppressed in arteriolar myocytes after SAH. However KV channel subtype expression was not changed. Our previous work demonstrated that the blood component oxyhemoglobin causes KV current suppression through heparin binding EGF-like growth factor (HB-EGF). HB-EGF caused significant K<sup>+</sup> current suppression in myocytes from control animals, but not after SAH. Further, HB-EGF suppressed K<sup>+</sup> currents in the absence and presence of the KV2 channel blocker stromatoxin, but failed to alter currents in the presence of 4-AP or correolide. These data suggest SAH causes KV1 current suppression through HB-EGF shedding, while KV2 current suppression is independent of the HB-EGF pathway. Supported by NIH P01 HL095488, Totman Medical Research Trust and the Peter Martin Fund.

**ROS-dependent and -independent MMP activation leads to KV current suppression in cerebral artery myocytes after SAH**

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Voltage-gated potassium (KV) channels regulate cerebral artery tone and are involved in pathologies associated with aneurysmal subarachnoid hemorrhage (SAH). We have previously demonstrated that matrix metalloprotease (MMP) activation by oxyhemoglobin (OxyHb) leads to epidermal growth factor receptor (EGFR) activation, KV current suppression and cerebral artery constriction. Here, we examined if enhanced MMP activity contributes to KV current suppression and cerebral artery constriction after SAH. Using whole cell patch clamp electrophysiology, we observed decreased KV currents in freshly isolated cerebral artery myocytes from SAH model rabbits. The following observations are consistent with enhanced MMP and EGFR activity involvement with SAH-induced KV current suppression: 1) OxyHb or the EGFR ligand, HB-EGF, failed to induced KV current suppression after SAH, and 2) gelatin zymography detected increased MMP-2 activity in cerebral arteries from SAH animals. Our data also suggest that OxyHb and SAH act through the same pathway (MMP and EGFR activation) to suppress KV currents. Reactive oxygen species (ROS) are generated in the auto-oxidation process of OxyHb, and are known to activate a variety of MMPs. To examine the mechanism of OxyHb-induced MMP-2 activation, zymography and KV current suppression were examined under conditions to minimize ROS. These studies indicate that OxyHb causes KV current suppression through ROS-dependent and ROS-independent pathways involving MMP activation. The ROS-independent pathway involves activation of MMP-2, whereas the ROS-dependent pathway involves activation of a second unidentified MMP. Further exploration of these mechanisms should reveal additional therapeutic targets for the treatment SAH. Supported by the NIH (P01-HL095488, R01-HL078983, R01-HL078983-05S1, P30 RR032135, P30 GM103498), The Totman Medical Research Trust and The Peter Martin Aneurysm Endowment.

**Contribution of Rho Kinase to TRPM4-Mediated Myogenic Tone in Cerebral Parenchymal Arterioles**

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Cerebral parenchymal arterioles (PAs) play a critical role in assuring appropriate blood flow and perfusion pressure within the brain. PAs are unique in contrast to cerebral pial arteries, as defined by their critical roles in neurovascular coupling and distinct sensitivities to mechanical and chemical stimulants. Elevation in intraluminal pressure, a physiologic vasomotor stimulant, causes smooth muscle membrane depolarization and contraction (myogenic tone) in cerebral arteries. However, the mechanisms by which this myogenic tone is regulated in the cerebral microcirculation remain unclear. Prior research in our laboratory indicates that myogenic tone of PAs is mediated primarily through stimulation of mechano-sensitive P2Y purinergic receptors. The objective of the present study was to determine the roles of TRPM4 channels and Rho kinase signaling in PA myogenic tone. We show that suppression of TRPM4 channel expression in vivo using antisense oligonucleotides reduced (by ~40%) both myogenic and P2Y-receptor agonist-induced tone in PAs. Further, we found that the Rho kinase inhibitor H1152 reversibly and nearly completely inhibited myogenic tone in these brain arterioles (IC<sub>50</sub>:0.2  $\mu$ mol/L). H1152 (1  $\mu$ mol/L) also attenuated P2Y<sub>4</sub> and P2Y<sub>6</sub> receptor-mediated vasoconstriction by 85% and 87%, respectively. Finally, H1152 (1  $\mu$ mol/L) reduced constitutive and UTP $\gamma$ S (0.5  $\mu$ M)-activated TRPM4 currents in parenchymal myocytes by 61% and 75%, respectively. These results indicate an important role for Rho kinase in regulation of TRPM4 channel activity and myogenic tone in the cerebral microcirculation.

### Impairment of Neurovascular Coupling by Chronic Stress

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Chronic stress (CS) is a contributory factor in a wide range of diseases. To date, no studies have focused on the effects of chronic stress on neurovascular coupling (NVC). NVC matches neuronal activity with an increase in local blood flow, ensuring that the metabolic demands of the active tissue are satisfied.

We studied NVC in a rat model of CS. Rats were exposed to one of 5 stressors each day for 7 days; we have previously shown that this produces an anxious behavioral phenotype. NVC was impaired by CS: vasodilation of parenchymal arterioles evoked by electrical field stimulation in brain slices was greatly reduced, whereas evoked astrocyte endfoot  $[Ca^{2+}]$  was enhanced. In isolated amygdalar arterioles of CS rats, dilation evoked by increasing  $[K^+]_o$  was diminished, suggesting an impairment of inward-rectifier  $K^+$  (Kir) channels. In myocytes from CS rats we observed a decrease in Kir current density. Corticosterone delivery produced a similar NVC phenotype to CS, suggesting that this molecule might mediate NVC impairment. These data suggest that CS causes a decrease in Kir channel number in myocytes of amygdalar parenchymal arterioles, possibly through the actions of corticosterone, rendering the vessel less able to respond to small increases in  $[K^+]_o$  released from astrocyte endfeet and resulting in impaired vasodilation after neuronal activity. This impairment may contribute to CNS disorders with a stress component.



**The increased amplitude of spontaneous Ca<sup>2+</sup> events in astrocytic endfeet parallels the inversion of neurovascular coupling after subarachnoid hemorrhage**

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The substantial mortality and morbidity associated with aneurysmal subarachnoid hemorrhage (SAH) can be attributed to the emergence of focal ischemic neurological deficits in the days to weeks following the initial bleed. Emerging evidence suggests that dysfunction of the intra-cerebral microcirculation contributes to the development of these deficits by restricting blood flow within the brain. Physiologically, brain function and metabolism are critically supported by a process, called neurovascular coupling (NVC), which matches focal increases in neuronal activity with local arteriolar dilation. Recently, an inversion of the NVC response, from vasodilation to vasoconstriction, was demonstrated in brain slices obtained from SAH model rats 4 days post-SAH. The evidence suggested that the increased amplitude of spontaneous Ca<sup>2+</sup> events in surrounding astrocytic endfeet caused this switch in the polarity of the vascular response by elevating perivascular K<sup>+</sup>. Therefore, to test this model we measured the NVC response and spontaneous Ca<sup>2+</sup> activity in astrocytic endfeet at 7 different time-points after SAH. Our data show that the inversion of NVC began within 24 hours of SAH and peaked at 2 days. Further, almost all time-points showing inversion of NVC also showed an increase in the amplitude of spontaneous Ca<sup>2+</sup> events in astrocytic endfeet. Importantly, these events occurred independent of neuronal activity and were due to Ca<sup>2+</sup> release from internal stores. While our data demonstrate a tight correlation between these phenomena, future studies will directly examine the role of the spontaneous Ca<sup>2+</sup> events in causing the inversion of NVC after SAH

**TRPV4 Ca<sup>2+</sup> sparklets in myoendothelial projections (MEPs) regulate vascular function**

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Endothelial cells (ECs) lining blood vessels regulate vascular tone. MEPs connect ECs to adjacent smooth muscle cells (SMCs) via gap junctions. We discovered elementary Ca<sup>2+</sup> signals (“sparklets”) through single TRPV4 channels at MEPs (Sonkusare et. al., Science, 2012). TRPV4 sparklets dilate 3rd order mouse mesenteric arteries (MAs) via EC intermediate-conductance, Ca<sup>2+</sup> sensitive K<sup>+</sup> (IK) channels. The current study elucidates the mechanism by which muscarinic receptor stimulation dilates MAs via TRPV4 channels. Carbachol (CCh) increased sparklets (5-fold) ONLY at MEPs, and not elsewhere on the EC membrane. Protein kinase C (PKC) activation similarly increased sparklets only at MEPs. CCh-activation of sparklets was prevented by PKC inhibitor Go-6976. Inward rectifier K<sup>+</sup> (Kir) currents were present in ECs but not in SMCs. Dilations to CCh, TRPV4 agonist GSK1016790A, and IK/SK opener NS309 were inhibited (70%) by Kir channel blocker Ba<sup>2+</sup>. The results support a MEP-localized signaling circuit of PKC-TRPV4-IK-Kir channels which mediates vasodilatory input to the SM.

**Activation of ATP-Sensitive Potassium (KATP) Channels underlies vasodilation to PACAP, but not CGRP, in Pressurized Rat Middle Meningeal Artery**

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Migraine is a complex neurological disorder that often presents as an intense unilateral headache accompanied by nausea, photophobia and other neurological symptoms. Activation of the trigeminovascular system and/or the sphenopalatine ganglia involving the release of the neuropeptides pituitary adenylate cyclase activating polypeptide (PACAP) and calcitonin gene related peptide (CGRP) has been implicated in vasodilation of the middle meningeal artery (MMA) and the sensation of migraine headache. However, the mechanism by which these two peptides exert their vasodilatory effect on the MMA is unclear. Activation of distinct receptors for PACAP and CGRP have been linked to activation of adenylyl cyclase in vascular smooth muscle. In addition, CGRP receptors have also been identified in vascular endothelial cells. Activation of cyclic AMP-dependent protein kinase has been shown to induce vasodilation via multiple mechanisms including phosphorylation and activation of smooth muscle KATP channels in a variety of vascular beds. In the present study our goal is to determine the role of KATP channels in vasodilation mediated via PACAP and CGRP in rat MMA. In isolated, pressurized MMAs both PACAP and CGRP induced significant vasodilation, although PACAP (EC<sub>50</sub> ~ 1 pM) exhibited ~ 1,000-fold greater potency compared to CGRP (EC<sub>50</sub> ~ 1 nM). PACAP-induced MMA dilation was completely abolished by the KATP channel inhibitor, glibenclamide (10 μM). In marked contrast, glibenclamide did not influence MMA dilation caused by CGRP. Further, N-Nitro-L-Arginine (L-NNA), a nitric oxide synthase inhibitor, had no effect on dilation caused by PACAP or CGRP. These observations demonstrate that PACAP dilates MMA via activation of vascular KATP channels, while CGRP acts through an alternative pathway. Thus, it appears that PACAP and CGRP contribute to the etiology of migraine via two distinct mechanisms. Therapeutic approaches targeting a combination of both PACAP and CGRP may be more effective than targeting either of these peptides alone in alleviating migraine headache. This work was supported by the Totman Medical Research Trust Fund, the Peter Martin Brain Aneurysm Endowment and the NIH (P01 HL095488, P30 RR032135 and P30 GM103498).

**A Decade of the Journal IMPULSE: Growth and Impact**

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The online, neuroscience journal for undergraduates, IMPULSE, was created in 2003 to fulfill a need for training in scientific publishing and peer review. There are a total of nine issues that have been archived annually, the tenth is the current, 2013 issue. It is an online international journal for research reports and reviews from undergraduate neuroscientists. While primary research expectations of undergraduate (pre-graduate, post-secondary school) education programs have increased, work completed by undergraduates is still usually not published or is compiled into a study submitted by the mentor. IMPULSE is intended to give students the opportunity to submit their neuroscience research so that they can experience the entire research process, including publication. Furthermore, undergraduate students may not be exposed to the peer review process, an important component of scientific publishing. IMPULSE also provides a mechanism for students to learn about the reviewing, editing and publishing side of research as well through reviewer training sites. The current report summarizes a survey of undergraduate experiences with IMPULSE over the last 10 years and demonstrates that IMPULSE is a useful teaching tool. It is an option for students who do not have the time or opportunity to do laboratory research and may serve as a means for faculty to provide some level of authentic research experience, at least on the publication side of science.

**COBRE NEUROSCIENCE CELL AND MOLECULAR BIOLOGY CORE FACILITY**

Sheryl White, Cindy Forehand and Rodney Parsons  
Department of Neurological Sciences

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, Northern, 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, Qiagen's QIAcube and a Qiagility liquid handling system.

**Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) in the Amygdala: Origin and Coexpression**

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In the central nervous system, pituitary adenylate cyclase activating polypeptide (PACAP) signaling is plays a role in stress, pain, and other emotion-related processes. PACAP-expressing fibers are abundant in the central amygdala, a site of integration between sensory and limbic pathways. Evidence suggests that PACAPergic fibers here may originate from cell bodies outside of the amygdala, however the location of these neurons is currently unknown. One potential candidate is the parabrachial nucleus (PBn) as PACAP-expressing cell bodies are found in the lateral PBn. This region is known to have projections to the central amygdala, some of which express calcitonin-gene related peptide (CGRP). These neurons are part of the spino-parabrachial-amygdaloid pathway that is implicated in the emotional responses to pain. In the amygdala this nociceptive pathway is thought to converge with fear and anxiety pathways. We hypothesize that PACAP-containing fibers in the amygdala originate from neurons in the lateral PBn as part of this pathway. We first examined PACAP and CGRP expression using double- labeling immunohistochemistry and found that they are coexpressed. To investigate the contribution of the PBn, a unilateral lesion in the lateral PBn was made and resulted in a loss of PACAP and CGRP expression in the amygdala. Additionally, anterograde neuronal tracer infused into the PBn overlaps with PACAP-expressing fibers in the amygdala. Finally to examine a role in pain behavior, PACAP-38 was infused into the central amygdala and lead to increased thermal sensitivity. These studies further substantiate a role for PACAP in the central integration of emotionally relevant sensory information.

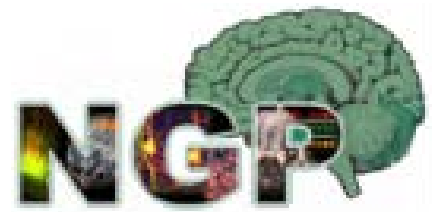
**The Ubiquitin Ligase Trim32 as a Regulator of Potassium Channels in the Brain**

Gene Cilento, Neuroscience Graduate Student, Department of Pharmacology/Neurological Sciences

Dynamic regulation of the voltage gated potassium channel Kv1.2 strongly influences neuronal excitability. Ubiquitylation, particularly monoubiquitylation, is one means of signaling such regulation by tagging ion channels for nondegradative, endocytosis. We used mass spectrometry (MS) to identify ubiquitylation sites within Kv1.2 purified from the brain. Additionally, MS analysis of Kv1.2 interacting proteins identified the ubiquitin ligase Trim32. *In vitro*, Trim32 was able to ubiquitylate Kv1.2 directly, supporting Trim32 as the source of Kv1.2 ubiquitylation in the brain. In cultured cells, Trim32 modulates Kv1.2 surface trafficking through mechanisms that either involve or are independent of ubiquitylation, depending on growth conditions. Additionally, overexpression of Trim32 alters the phosphorylation state of Kv1.2, proposing a complex model of Kv1.2 modulation that likely involves cross-talk between post-translational modifications. Altogether, our study demonstrates a new mechanism for the regulation of Kv channels in the brain and provides new insight towards neuronal excitability control.

## Sponsors

Financial support for this meeting was provided by generous donations from the following sources:



## Acknowledgements

We would like to thank the Vermont Chapter of the Society of Neuroscience officers and representatives for helping with the organization and planning of this meeting.

We would also like to thank the members of the NBH Steering Committee for their support.