THIRD ANNUAL NEUROSCIENCE, BEHAVIOR AND HEALTH RESEARCH FORUM

The University of Vermont
Davis Center Grand Maple Ballroom
January 18-19, 2013

Presented by:

NEUROSCIENCE, BEHAVIOR AND HEALTH RESEARCH FORUM
SCHEDULE OF EVENTS

FRIDAY, JANUARY 18

3:00 Poster setup

4:00 Registration, Posters and Refreshments

5:00 Introductory Remarks: George Wellman, PhD, President, Vermont Chapter of the Society for Neuroscience

5:15 Keynote presentation: **James Herman**, **PhD**, Professor of Psychiatry and Behavioral Neuroscience and Director of the Laboratory of Stress Neurobiology, University of Cincinnati
Title: ***Rethinking Stress Biology***

6:00 Reception

SATURDAY, JANUARY 19

8:00 – 8:30 Poster setup

8:30 – 9:30 Posters

9:15 Introductory Remarks: Domenico Grasso, PhD, PE, DEE, Vice President for Research and Dean of the Graduate College and Rae Nishi, PhD, Director of Neuroscience, Behavior and Health Initiative, University of Vermont

Session I: Chaired by Stephanie Spohn/Abbie Chapman

9:30 Hugh Garavan: *Prediction Inflation in Neuroimaging Studies*

9:50 Robert Whelan: *Classification and prediction of alcohol misuse in an adolescent sample*

10:05 Jane Kolodinsky: *A behavioral economic consideration of the impacts of a sugar sweetened beverage tax on demand for sugar sweetened beverages in Vermont and cross-border shopping for sugar sweetened beverages*

10:25 Alice Schermerhorn: *Stress and child development: individual differences in vulnerability*

10:45 Coffee and snacks

Session II: Chaired by *Galen Missig/Gene Cilento*

11:00 Diane Jaworski: *Dietary acetate supplementation as a novel therapeutic approach to eradicate glioma stem-like cells*

11:20 Mathew Lecomte: *Endothelin receptor B as a potential regulator of neural progenitor cell migration from the subventricular neural stem cell niche*

11:35 Lindsay Naef: *Blame it on your Mom! Long-term consequences of early exposure to high fat on mesolimbic dopamine function*

11:50 Nathan O'Connor, MBF Bioscience: Latest advancements in whole slide imaging, automated neuron tracing, and *C. elegans* tracking from MBF Bioscience

12:05-2:00 Lunch and posters

Session III: Chaired by **Patrick Long/Eric Gonzalez**

2:00	Anthony Pappas: *High amplitude spontaneous Ca^{2+} events in astrocytic endfeet may underlie the inversion of neurovascular coupling after subarachnoid hemorrhage*
2:15	Thomas Longden: *Impairment of Neurovascular Coupling by Chronic Stress*
2:30	Liana Merrill: *Role of vanilloid transient receptor potential cation channel (TRPV) 4 in bladder dysfunction in response to repeated variate stress (RVS) in male rats*
2:45	Break

Session IV: Chaired by Dave Harris/Geoff Schaubhut

3:00	Vanessa Ochoa: *A newly identified prototoxin, LYPD6B, modulates the function of the alpha3 beta4 nicotinic acetylcholine receptor*
3:15	Olga Lipatova: *The role of estrogen in cognition, behavioral flexibility and serotonin 6 receptor*
3:30	Greg Lieberman: *Changes in white matter as a measure of neuroplasticity following cognitive behavioral therapy for coping with chronic pain*
3:45	**Awards and closing remarks**

NEUROSCIENCE, BEHAVIOR AND HEALTH RESEARCH FORUM
POSTER SESSION

Poster #1 **Rnai-Mediated Knockdown Of Nav1.1 Disrupts A Cognitive Neural Network**
AC Bender, SV Campos, H Natola, BW Luikart, RC Scott, GL Holmes, PP Lenck-Santini

Poster #2 **The COBRE Imaging And Physiology Core: A Central Resource For High Resolution Live Imaging**
Todd A. Clason and Cindy J. Forehand

Poster #3 **Constitutively Active Trkb Confers An Aggressive Transformed Phenotype To A Neural Crest Derived Cell Line**
John DeWitt PhD, Vanessa Ochoa BS, Johann Urschitz PhD, Marlee Elston BS, Stefan Moisyadi PhD, Rae Nishi PhD

Poster #4 **TRPV4 Channels Tune Astrocyte Endfoot Ca2+ To Optimize Neurovascular Coupling**
Kathryn Dunn and Mark T. Nelson

Poster #5 **Cavernous Nerve Dissection And Stretch Without Cutting Or Crushing Decreases Transcript Levels For The Neuronal Nicotinic Acetylcholine Receptor (Nachr) Subunit A3 And Postsynaptic Density (PSD)-93 In Male Mouse Major Pelvic Ganglia (MPG)**
Beatrice M. Girard, John Tompkins, Margaret A. Vizzard, Rodney L. Parsons

Poster #6 **Plasticity In Transient Receptor Potential (TRP) Channel Expression In Urinary Bladder In Rodents With Urinary Bladder Dysfunction Or During Postnatal Development**
Beatrice M. Girard, Susan Malley, Liana Merrill, Margaret A. Vizzard

Poster #7 **Co-Localization Of The Lynx1-A Prototoxin And A7 Nicotinic Acetylcholine Receptors In Human Embryonic Kidney 293 Cells**
Chelsea Manning and Felix Eckenstein

Poster #8 **TRPC3 Channel Regulation Of Sensory Afferent Extension In The Embryonic Avian Spinal Cord**
M. McNamara, T. Clason, C.J Forehand

Poster #9 **Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) In The Amygdala: Origin And Coexpression**
G. Missig, C.W. Roman, K.M. Braas, S.E. Hammack, V. May

Poster #10 **Colocalization Of The Prototoxin Prostate Stem Cell Antigen With α7 Nicotinic Acetylcholine Receptors And Acetylcholinesterase In Parasympathetic Neurons**
Simone Otto, Richard L. Rotundo, Rae Nishi

Poster #11 **Detection Of L-Amino Acids Involves Taste Receptors In Addition To T1R1/T1R3**
Shreoshi Pal Choudhuri, Rona J. Delay, Eugene R. Delay

Poster #12 **Intra-Bnst Pituitary Adenylyl Cyclase Activating Polypeptide Increases Plasma Corticosterone**
E. Roelke, K. R. Lezak, O. Harris, G. Cocchiaro, G. Missing, C. W. Roman, K. M. Braas, V. May, S. E. Hammack

Poster #13 **ATP Excites Mouse Vomeronasal Sensory Neurons Through Activation Of P2X Receptors**
Jonathan Vick and Rona Delay

Poster #14 **Cobre Neuroscience Cell And Molecular Biology Core Facility**

Sheryl White, Rae Nishi, Cindy Forehand, Rodney Parsons

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Meghan C. Eddy, Kate J. Stansfield, John T. Green

Poster #16 **The Effects Of Secretin Infusions Into Different Areas Of Cerebellar Cortex On Rat Eyeblink Conditioning**
J. R. Fuchs, W. Gove, J. T. Green

Poster #17 **Exercise And Subchronic Fluoxetine Produce A Reduction In Anxiety In C57 Mice That Is Greater Than Exercise Or Fluoxetine Alone**
B.D. Hare, S. A. Hammack, J.H. Fox, W. A. Falls

Poster #18 **Different Subregional Effects Of Intra-BNST PACAP On Stress And Anxiety-Like Behavior**
O. Harris, M. Kocho-Schellenberg, K. R. Lezak, C. W. Roman, M. J. Hartsock, R. Kiefer, E. A. Wasserman, S. E. Hammack

Poster #19 **Working Memory Ability Modulated Brain Functional Connectivity During Dopamine Agonist Stimulation In Postmenopausal Women**
Jenna Makarewicz, Robert Devins, Magdalena Naylor, Julie Dumas

Poster #20 **Adults With ADHD Show Reduced Prefrontal Cortex And Subcortical Activation In A Fmri Delay Discounting Task**
Nick Ortiz, Robert Whelan, K L Brennan, Redmond O'Connell, Jessica Bramham, Hugh Garavan

Poster #21 **Burn And Earn: A Randomized-Controlled Trial Examining The Efficacy Of Incentives To Motivate Fitness-Center Attendance In College Freshmen**
Lizzy Pope, MS, RD and Jean Harvey-Berino, PhD, RD

Poster #22 **Characterizing PACAP Receptors Involved In Anxiety-Like Responses: Evidence For HPA-Axis Activation By BNST PACAP And PAC1 Receptor Signaling**
C. W. Roman, G. Missig, K. R. Lezak, M. Kocho-Schellenberg, K. M. Braas, S. E. Hammack, V. May

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Geoffrey Schaubhut, Emily C. Mazzulla, Sarahjane L. Dube, Alexandra S. Potter

Poster #24 **Cerebellar Contributions To Impulsivity Phenotypes**
Eli Sepkowitz, Geoffrey Schaubhutt, John Green, Alexandra Potter

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K Weierstall, R Whelan, H Garavan

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Gregory Lieberman, Richard Watts, Trevor Andrews, Marina Shpaner, Christopher Filippi, Magdalena Naylor

Poster #27 **Anatomical Changes Following Cognitive Behavioral Therapy In Patients With Chronic Musculoskeletal Pain**
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Katherine Balas, Jenna Makarewicz, Robert Devins, Peter Casson, Magdalena Naylor, Paul Newhouse, Julie Dumas

Poster #29 **5HT4 Receptors In The Colon And Duodenum Mucosa Educes 5-HT Release**
Amanda F. Bolgioni, Brigitte Lavoie, Gary M. Mawe

Poster #30 **The Neurobiology Of Successful Heroin Abstinence**
KL Brennan, Robert Whelan, Nick Ortiz, Katriona O Sullivan, Adam Stone, Hugh Garavan

Poster #31 **Natural Variation In The Murine Y Chromosome Influences Gene Regulation And Susceptibility To Experimental Allergic Encephalomyelitis**
Laure K. Case, PhD, Emma H. Wall, PhD, Julie Dragon, PhD, Naresha Saligrama, MVSc, James F. Zachary, DVM, PhD, Elizabeth P. Blankenhorn, PhD, Cory Teuscher, PhD

Poster #32 **An Enhanced Myogenic Vasodilatory Response To Hypotension In Posterior Cerebral Arteries Of Pregnant Rats Is Nitric Oxide Dependent**
Abbie C Chapman, Siu-Lung Chan, Marilyn J Cipolla

Poster #33 **Profound Decrease In Myogenic Tone Of Parenchymal Arterioles In A Genetic Model Of Cerebral Ischemic Small Vessel Disease**
Fabrice Dabertrand, Adrian D Bonev, Jill E Ingalls, Joseph E Brayden, Anne Joutel, Mark T Nelson

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E. Gonzalez, B. M. Girard, S. Malley, M. A. Vizzard

Poster #35 **The Effect Of Cyclophosphamide On C-Fos-Like Labeling In The Nucleus Of The Solitary Tract**
Christopher Stecyk, David Harris, Eugene Delay

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Nathan Jebbett, Joshua Hamilton, Matthew D. Rand, Felix P. Eckenstein

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Poster #38 **P38 Map Kinase Signaling In Myeloid Cells Controls Autoimmune Disease Of The Cns In A Sex-Specific Manner**
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Yao Li and Joseph Brayden

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Arsalan U. Syed, Masayo Koide, Victor May, George C. Wellman

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Thomas J. Heppner, Nathan R. Tykocki, Gerald C. Mingin, Mark T. Nelson

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ABSTRACTS

PLATFORM TALK ABSTRACTS

Listed by order of presentation

Prediction Inflation in Neuroimaging Studies

Hugh Garavan & Robert Whelan

Departments of Psychiatry and Psychology, University of Vermont, USA

Much of the excitement surrounding non-invasive brain imaging arises from its potential to reveal a mechanistic understanding of neural function in health and disease. Armed with this knowledge, researchers may be able to identify and predict important outcomes in numerous clinical domains such as which teenagers will transition to psychosis, which adults will transition to Alzheimers disease, which depressed patients will respond to treatment and which abstinent drug users will relapse. In this presentation, we will demonstrate that the typical approach in this field, identifying predictors based on group comparisons in which the outcome is known, overestimates the true predictive ability of neuroimaging measures, by making one or both of two types of error. First, comparing groups predicated on a known outcome can inflate prediction estimates by misrepresenting the base rate of the outcome of interest. Second, predictive models are often overfit to the tested samples and consequently fail to generalize to new data. We will show via simulated data that models can appear to be predictive, even when the data and outcomes are entirely random, and that even moderate effect sizes can appear almost perfectly predictive. We note that the disparity between retrospective prediction (postdiction) and prediction is especially pernicious in domains in which the number of possible predictors is high (e.g., multi-voxel analyses or genome-wide association studies that compare groups that vary on a known phenotype), that it persists even if the postdicted effects are highly significant, and we suggest a number of approaches, adopted from machine learning, to quantify prediction accurately.

Classification and Prediction Of Alcohol Misuse In An Adolescent Sample

Robert Whelan, Hugh Garavan, and the IMAGEN Consortium

Department of Psychiatry, Department of Psychology, University of Vermont

We employed a machine learning approach – logistic regression with elastic net regularization – to both classify 14-year-old subjects as either current alcohol misusers or as low alcohol controls and also to predict which currently non-alcohol-using subjects at age 14 would misuse alcohol at age 16. Both models incorporated the following features: structural and functional brain data, personality measures, parental substance use, nicotine use, and selected genetic information. To classify current drinkers we selected subjects with three lifetime binge drinking episodes at age 14 (n=192) and compared them to subjects with fewer than two lifetime drinks until at least the age of 16 (n=157). The model classified subjects with high accuracy on novel data: area under the curve (AUC) of the receiver-operator characteristic = 0.902. Gray matter structure, parental substance use, and personality variables contributed more highly to the model than functional brain data, and genetic data were excluded from the final model. To predict future drinkers, all participants (n=272) had zero-to-low drinking at baseline (fewer than 2 lifetime drinks). At age 16, 151 participants remained at baseline levels of drinking, whereas 121 participants had at least three lifetime binge drinking episodes. The predictive model was moderately accurate on novel data (AUC = 0.692) and placed greater emphasis on the functional and structural brain data than the baseline classifier, with reduced weighting for personality and parental substance use, and genetic data were again excluded. The present study may be the first to develop a predictive model of substance misuse in adolescence.

A Behavioral Economic Consideration Of The Impacts Of A Sugar Sweetened Beverage Tax On Demand For Sugar Sweetened Beverages In Vermont And Cross-Border Shopping For Sugar Sweetened Beverages

Jane Kolodinsky[1], Richard Watts[1], Rachel Johnson[2], Michael Moser[3], Sarah Heiss[1]

[1]Dept of Community Development and Applied Economics, [2]Dept of Nutrition & Food Science, [3]Center for Rural Studies, University of Vermont

The authors investigated whether a proposed penny per ounce sugar sweetened beverage (SSB) tax would cause decreased SSB demand or promote cross border shopping. We used survey data and difference in difference modeling to examine associations between SSB demand, a SSB excise tax, border county residence, and demographic characteristics.

A SSB excise tax would reduce SSB demand. There was no evidence that a penny per ounce SSB excise causes smaller and border county retailers to suffer lower sales compared with other retailers. Results suggested that lower income consumers and overweight/obese consumers do not have a different price sensitivity compared to higher income and healthy weight consumers. For all model specifications, only the tax coefficient was significant. Calculated elasticities were within ranges of other studies, between -.71 and -2.0.

This study suggests that the economic and cross border shopping arguments opposing SSB excise taxes as a policy instrument to help decrease the obesity problem may not have a strong empirical basis.

Stress And Child Development: Individual Differences In Vulnerability

A. Schermerhorn

Department of Psychology, University of Vermont

Stress is associated with child maladjustment, but the association is not especially strong, suggesting that other factors, like temperament characteristics, might elevate risk of problems. We investigated the interaction between two temperament characteristics (high vs. low resistance to control [unmanageability] and fearfulness [novelty distress]) and family stress in predicting externalizing problems in 556 children followed from kindergarten through eighth grade (ages 5–13). The combination of high resistance to control and high fear strengthened the stress–externalizing association. The next step for research in this area is to investigate underlying mechanisms, such as neural processes, that might account for these associations. We will describe our approach to examining such underlying mechanisms using event-related potential methods, and provide a preliminary graphical depiction of data from two representative children in our initial pilot study, one scoring high in temperament-related fearfulness, and the other scoring low in fearfulness (top/bottom 20%).

Dietary Acetate Supplementation As A Novel Therapeutic Approach To Eradicate Glioma Stem-Like Cells

Patrick M. Long[1#], Andrew R. Tsen[2#], Matthew T. Davies[1], Benjamin A. Teasdale[1], John R. Moffett[3], Aryan M. A. Namboodiri[3], Scott Tighe[4], Wai Lam[4], Paul L. Penar[2], William W. Pendlebury[6], Jeffrey L. Spees[7], Sean E. Lawler[8], Mariano S. Viapiano[9], and Diane M. Jaworski*[1].

[1]UVM Department of Neurological Sciences; [2]UVM Department of Surgery, Division of Neurosurgery; [3]Department of Anatomy, Physiology & Genetics, Uniformed Services University of the Health Sciences, Bethesda, MD; [4]UVM Vermont Genetics Network; [6]UVM Department of Pathology; [7]UVM Department of Medicine; [8]Leeds Institute of Molecular Medicine, St. James Univ. Hospital, Leeds,UK; [9]Department of Neurosurgery, Brigham and Women's Hospital, Boston MA. # Denotes equal contribution

Glioma, the most common primary brain tumor of the adult central nervous system, is associated with a poor prognosis (median survival of 14 months) due, in part, to chemo-radiotherapy-resistant glioma stem-like cells (GSCs) that are responsible for post-surgical recurrence. N-acetyl-L-aspartate (NAA), the most concentrated metabolic source of acetate in the brain, and aspartoacylase (ASPA), the enzyme responsible for NAA degradation, are significantly reduced in glioma, thereby reducing acetate bioavailability. NAA-derived acetate is converted to acetyl coenzyme A (acetyl-CoA) for use in lipogenesis, protein/histone acetylation, and the TCA cycle. We propose that glyceryltriacetate (GTA), a FDA approved food additive with “generally regarded as safe” status, may be an effective means of reducing glioma growth via restoration of acetate levels.

The effect of GTA on the growth of grade II & grade III oligodendroglioma, and grade IV astrocytoma (GBM) GSCs was assessed. *In vitro*, GTA induced cytostatic growth arrest, rather than cytocidal apoptosis, on all GSCs, but had no effect on neural stem cells. Interestingly, GTA induced a novel 26 kDa ASPA isoform in several of the GSCs and preliminary data indicates this isoform partitions exclusively to the nucleus. ASPA and acetyl-CoA synthetase co-localized in the nucleus and GTA increased protein acetylation, including histone H4, suggesting an epigenetic mechanism underlying GTA-mediated growth arrest. Finally, the effect of GTA on orthotopically grafted luciferase expressing GSCs was assessed. GTA synergized with temozolomide (TMZ) chemotherapy to decrease oligodendroglioma tumor bioluminescence and volume and increase survival relative to TMZ alone. More strikingly, GTA alone increased survival of mice engrafted with the most aggressive GBM GSC line. Inasmuch as infants with Canavan Disease, a leukodystrophy due to ASPA mutation, treated with high dose GTA showed no significant side effects, GTA may prove an effective therapy to prevent glioma recurrence by inducing growth arrest of GSCs.

Endothelin Receptor B as A Potential Regulator Of Neural Progenitor Cell Migration From The Subventricular Neural Stem Cell Niche

LeComte M*[1,2], Bibeau AL[2,3], Spees JL[1,2,3]

[1]Department of Neurological Sciences, [2]Department of Medicine, and [3]Stem Cell Core, University of Vermont

Neural Stem/Progenitor Cells (NSCs/NPCs) are multipotent cells with potential to generate replacement cells for injured tissue after CNS injuries such as stroke. In most cases, however, NPCs do not migrate from the subventricular zone (SVZ) niche in numbers sufficient to replace the vast number of cells destroyed during stroke. The identification of migratory cues for NPCs may provide information regarding receptor ligands or other molecules through which we can improve CNS repair after injury. We recently determined that a sub-population of cortical reactive astrocytes isolated from peri-infarct tissues after stroke could generate cortical NSCs (cNSCs) with many fundamental properties of bona fide NSCs/NPCs from the lateral ventricle. Similar to SVZ-NSCs, cNSCs could self-renew and differentiate into neurons, oligodendrocytes and astrocytes in culture. Notably, however, after transplantation into the lateral ventricles of healthy mice, cNSCs self-renewed in the SVZ but did not migrate in the rostral migratory stream (RMS) in the same manner as did age-matched SVZ-NSCs. Whereas SVZ-NSCs entered the RMS and reached the olfactory bulb within 1 month following transplantation, cNSCs required up to 3 months to reach the olfactory bulb. This observation suggested that cNSCs might respond differently to soluble migration cues regulating NPC exit from the SVZ niche. With microarray-based gene expression analysis of cNSC and SVZ-NSC clones (N=3), we identified Endothelin receptor type B (ETRB) mRNA as differentially expressed between the two sets of clones. Immunoblotting confirmed that SVZ-NSCs expressed higher levels of ETRB protein. By immunohistochemical assays, doublecortin-positive neuroblasts in the RMS expressed low or undetectable levels of ETRB. In contrast, NPCs immediately exiting the SVZ niche appeared to stain strongly for the receptor. Using lentiviral shRNA vectors, we have produced knockdown lines of SVZ-NSCs to examine the potential role of ETRB and Endothelin as a signaling cue that controls the NPC migration. Furthermore, we are currently generating CD133 (Prominin1)-ETRB conditional knockout mice to remove the Endothelin B receptor from NSCs residing in the SVZ niche. We anticipate that these mice will exhibit a defect in neuroblast migration and the formation of inhibitory interneurons in the olfactory bulb. In contrast, injection of ETRB agonist should increase neuroblast migration and identify the endothelin system as a potential means to alter NPC behavior after CNS injury.

Blame It On Your Mom! Long-Term Consequences Of Early Exposure To High-Fat On Mesolimbic Dopamine Function

Lindsay Naef, Luc Moquin, Alain Gratton, & Claire-Dominique Walker.

Integrated Program in Neuroscience, Douglas Mental Health University Institute, McGill University

Alterations in the nutritional and hormonal environment of developing young predispose individuals to the development of obesity in adulthood. We have previously demonstrated that exposure to high-fat during early development alters the presynaptic regulation of mesolimbic dopamine (DA) and increases incentive motivation for high-fat food rewards. In the present experiment, we test the hypothesis that early exposure to high-fat through the maternal milk programs nucleus accumbens (NAc) DA responses to food and food cues in the adult offspring. Mothers were maintained on a high-fat (30% fat) or control (5% fat) diet from gestation day 13 to postnatal day 22 when offspring from both diet groups were weaned and maintained on the control diet until testing in adulthood. In vivo NAc DA responses to food anticipation and consumption were measured in a Pavlovian conditioning paradigm using voltammetry in freely moving rats. We demonstrate that high-fat offspring display a reduction in their anticipatory, but not consumatory DA responses to food, suggesting that the increased operant responding to fat pellets in these rats is a consequence of maternally programmed DA hypofunction. These data demonstrate that exposure to high-fat during early development might program behavioral and functional responses associated with mesolimbic DA neurotransmission, thus leading to an increased high-fat feeding and obesity.

High Amplitude Spontaneous Ca^{2+} Events in Astrocytic Endfeet May Underlie the Inversion of Neurovascular Coupling after Subarachnoid Hemorrhage

Anthony C. Pappas[1], Masayo Koide[2], George C. Wellman[2]

[1]Department of Neurological Sciences, [2]Department of Pharmacology, University of Vermont

Dysfunction of the intra-cerebral microcirculation may contribute to the development of delayed ischemic neurological deficits following aneurysmal subarachnoid hemorrhage (SAH). Neurovascular coupling (NVC), which links focal increases in neuronal activity with local arteriolar dilation, is essential for proper brain function and metabolism. Recently, we reported an inversion of the NVC response in brain slices obtained from SAH model animals (Koide et. al. *PNAS* 2012). Rather than dilate, brain parenchymal arterioles constrict following neuronal activation. The evidence suggests that higher amplitude spontaneous Ca^{2+} events in astrocytic endfeet set the stage for the inversion of NVC by increasing the basal perivascular K^+ concentration. While this study determined a mechanistic link between altered Ca^{2+} activity of perivascular astrocytes and impaired neurovascular communication, it only examined animals 4 days after SAH induction. Using combined infrared differential interference contrast microscopy and 2-photon laser microscopy to image acute cortical brain slices, we examined the NVC response and spontaneous Ca^{2+} activity in astrocytic endfeet at six time-points after SAH. Our results show that the onset of the inversion of NVC occurs within 24 hr of SAH and coincides with an emergence of higher amplitude spontaneous Ca^{2+} events in astrocytic endfeet. Further, all time-points showing inversion of NVC also show a greater proportion of high amplitude spontaneous Ca^{2+} events. These data support a model in which altered Ca^{2+} signaling of astrocytic endfeet contributes to the NVC deficits observed after experimental SAH.

This work was supported by the Totman Medical Research Trust Fund, the NIH (P01 HL095488, P0195488-S1, P30 RR032135, and P30 GM103498), and the UVM Neuroscience COBRE Imaging Facility.

Impairment of Neurovascular Coupling by Chronic Stress

TA Longden[1], F Dabertrand[1], SE Hammack[2], MT Nelson[1]

[1]Department of Pharmacology, [2] Department of Psychology, University of Vermont

Chronic stress 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. One mechanism of NVC involves the release of K^+ ions from astrocyte endfeet, which cause vasodilation through activating myocyte K_{IR} channels.

We studied NVC in a rat model of chronic stress (CS), using varied stressors for 7 days, which produced an anxious behavioral phenotype. NVC was impaired in CS rats: vasodilation of parenchymal arterioles evoked by electrical field stimulation (EFS) 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^+ (K_{IR}) channels. In isolated myocytes from CS rats we observed a decrease in K_{IR} current density. In control brain slices 100 μM Ba^{2+}, a K_{IR} channel blocker, inhibited EFS-evoked NVC, whereas Ba^{2+} had no effect on the impaired vasodilations observed in CS rats. Similarly, raising $[K^+]_o$ to 10 mM substantially dilated amygdalar arterioles in control slices, but this effect was diminished in arterioles from CS rats.

Collectively, these data suggest that CS causes a decrease in K_{IR} channel density in myocytes of amygdalar parenchymal arterioles, 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.

Role of Vanilloid Transient Receptor Potential Cation Channel (TRPV) 4 in Bladder Dysfunction in Response to Repeated Variate Stress (RVS) In Male Rats

L. Merrill, B. Girard, A. Peterson, M. Vizzard

Department of Neurological Sciences, University of Vermont

Interstitial cystitis (IC)/bladder pain syndrome (BPS) is a chronic pelvic pain disorder with urgency, frequency, and suprapubic pain often exacerbated by stress. Multiple TRP channels are expressed in the bladder and may act as sensors of stretch and/or chemical irritation and may play functional roles in overactive bladder and IC/BPS. We have shown that RVS significantly increases voiding frequency and decreases bladder capacity and void volumes, as well as increases somatic sensitivity, in both male and female rats. We have now examined the role of TRPV4 in bladder function following RVS. We previously demonstrated increased NGF expression in bladder of stressed rats; therefore, TRPV4 mRNA expression was determined in a transgenic mouse with chronic NGF overexpression (OE) in the bladder. Stressed rats were exposed to a 7-day RVS paradigm with a single stressor presented daily. Control rats were handled but not exposed to stressors. Bladder function was evaluated with open outlet, conscious cystometry. Bladders were harvested from stressed and control rats and NGF-OE mice to determine TRPV4 mRNA bladder expression using quantitative real-time PCR. RVS significantly ($p \leq 0.01$) increased voiding frequency and decreased void volumes and bladder capacity. In stressed rats, intravesical administration of the TRPV4 antagonist HC067047 (1 μM) significantly ($p \leq 0.01$) increased bladder capacity (2.3-fold) and void volume and decreased voiding frequency (2.4-fold). TRPV4 mRNA expression was significantly ($p \leq 0.01$) increased in bladder from stressed rats and in NGF-OE mouse bladders. CVS alters normal urinary bladder function and blockade of TRPV4 improves bladder function following stress.

NIH Grant DK051369

NIH Grant DK060481

NIH Grant DK065989

National Center for Research Resources 5 P30 RR 032135

National Institute of General Medical Sciences 8 P30 GM 103498

A Newly Identified Prototoxin, LYPD6B, Modulates the Function Of The Alpha3 Beta4 Nicotinic Acetylcholine Receptor

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Signaling through nicotinic acetylcholine receptors (nAChRs) is involved in a variety of developmental processes, such as neural proliferation, differentiation, and survival as well as in neural communication. A family of proteins known as prototoxins has been identified as molecules involved in the modulation of signaling through nAChRs. In the chicken ciliary ganglion, we have identified a new member of the prototoxin family, LYPD6B. We hypothesize that the newly identified prototoxin LYPD6B binds to the alpha3beta4 nAChR, and as a consequence decreases acetylcholine evoked responses. End point RT PCR on isolated RNA shows LYPD6B to be expressed in chicken ciliary ganglion and brain but not stomach or lung tissues. To test the effect LYPD6B has on the alpha3beta4 nAChR, two electrode voltage clamp, intracellular recordings were performed on Xenopus oocytes. A concatemeric construct was used, in order to restrict the stoichiometry of the beta4alpha3beta4beta4alpha3 nAChR. The concatemer is designed so that all of the subunits are connected by linker regions and expressed as a single polypeptide chain; thus the stoichiometry of the nAChR is fixed. The Xenopus oocytes co-expressing the beta4alpha3beta4beta4alpha3 concatemer and LYPD6B exhibited decreased peak acetylcholine evoked current responses when compared to the group expressing the beta4alpha3beta4beta4alpha3 concatemer alone, but no response difference among the variety of acetylcholine concentrations. The results suggest LYPD6B to have a neural specific role that modulates the function of alpha3beta4 nAChR. Two future experiments will support the hypothesis that LYPD6B binds to alpha3beta4: immunocytochemistry (demonstrating co-localization) and a co-immunoprecipitation.

The Role of Estrogen in Cognition, Behavioral Flexibility and Serotonin 6 Receptor

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Numerous studies have shown that estrogen can either enhance or impair memory in female rats. It is possible that the type of experimental paradigm used to test the effect of estrogen on learning may contribute to the differential results. The present talk addresses the effect of estradiol (E2) on several elements of cognition in rats that were trained in a specific type of experimental setting. The findings showed that cycling-E2 treatment unlike chronic-E2 treatment facilitates acquisition of spatial memory in female rats that were trained in a safe experimental context. The findings also showed that both E2 treatment regiments enhance retention of spatial memory. However, E2 appears to disrupt the expression of spatial memory when rats were tested with a novel start location. This result may be due to the rats' impairment in behavioral flexibility induced by a sustained habitual response. Additionally, the present talk will present quantitative PCR data indicating that E2 modulates the expression of serotonin 6 receptor ($5HT_6R$) mRNA differentially in the dorsolateral striatum and hippocampus of female versus male rats. The $5HT_6R$ has been previously shown to play a role in behavioral flexibility. The regulation of this receptor by E2 in a sex and brain-region dependent fashion may be involved in the differential effects of E2 on place vs. response learning on a spatial task and the induced impairment caused by a novel condition. In conclusion, specific E2 regiments beneficially effect differential memory stages (acquisition vs. retention) and systems (hippocampus- vs. striatum-dependent memory), while impair behavioral flexibility through the modulation of $5HT_6R$.

Changes In White Matter As A Measure Of Neuroplasticity Following Cognitive Behavioral Therapy For Coping With Chronic Pain

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Cognitive Behavioral Therapy (CBT) has the ability to alter the ways in which we think, feel, behave, and perceive pain. Using functional Magnetic Resonance Imaging (fMRI), our lab has previously demonstrated that there are differences in neural function between the brains of healthy volunteers and chronic pain patients, and that these functional differences can be attenuated after as few as three months of CBT for coping with chronic pain. Further, gray matter volume significantly increases within functionally relevant brain regions after completion of CBT (in preparation for publication). We hypothesize that these changes in gray matter structure and function are accompanied by corresponding neuroplastic changes in the properties of white matter, as measured by Diffusion Tensor Imaging (DTI). Specifically, we expect to observe changes in fractional anisotropy (FA) within tracts that connect brain regions associated with perception of pain and that show structural and functional differences after CBT. We predict that these changes will also correlate with improvements in clinical outcomes.

POSTER ABSTRACTS

Listed by Poster Number

Poster #1

RNAi-Mediated Knockdown of Nav1.1 Disrupts a Cognitive Neural Network

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Background: Dravet syndrome (DS) is a childhood-onset epilepsy leading to severe and permanent cognitive impairment. As many as 85% of cases of DS are linked with loss-of-function mutations in the SCN1A gene, coding for the type I voltage-gated sodium channel (Nav1.1). However, the impact of Nav1.1 deficits on cognitive neural networks has not been studied. Here, we use an RNAi approach to investigate the direct effects of Nav1.1 downregulation on specific neural networks in vivo.

Summary of Results: We found that focal Nav1.1 downregulation in the basal forebrain region caused a spatial memory impairment. Continuous EEG monitoring revealed that this effect was not caused by seizures. Rather, the fundamental neurophysiological properties of this network were altered. Specifically, hippocampal theta frequency was associated with spatial memory performance but was reduced after Nav1.1 knockdown. Secondly, single-unit recordings of basal forebrain neurons in vivo demonstrated that the firing properties of this neuronal population were substantially impacted. The average peak firing frequency was reduced and the average action potential width was increased. These deficits may help explain the dysregulation of hippocampal oscillations and function. Our results to this point suggest that the loss of function of Nav1.1 in Dravet syndrome may directly impact cognition through mechanisms other than seizures.

Poster #2

The COBRE Imaging and Physiology Core: A Central Resource for High Resolution Live Imaging

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The University of Vermont COBRE Imaging and Physiology Core is a central resource which offers a wide range of imaging hardware for fixed and live-cell immunofluorescence studies. We maintain a Noran/Prairie Technologies confocal microscope, a Nikon TIRF system, a PTI monochromator system with a Photometrics Evolve EMCCD, a DeltaVision restoration microscopy system, a Zeiss/BioRad dedicated multiphoton microscope, an Andor iXon/Olympus widefield fluorescence microscope, and a Nikon SMZ-1500 fluorescence stereoscope. Patch-clamp and cell stimulation equipment is available for use on most microscope systems. The Noran is capable of high-speed acquisition, and is used primarily for live calcium imaging on whole tissues and isolated cells. The DeltaVision system has a temperature and humidity-controlled incubator allowing for time-lapse experiments on cultured cells, and also excels at immunofluorescence and colocalization experiments on fixed samples. The BioRad Radiance 2100 Dedicated Multiphoton includes a Coherent Chameleon 210 nm femtosecond pulsed IR laser mounted on a fixed-stage upright Olympus microscope. The pulsed infrared excitation, along with non-descanned detectors, makes it possible to image at depth in live brain slices to study neurovascular coupling. The PTI ratiometric system can visualize calcium flux using a wide variety of Calcium indicators, including, Fura-2AM, Fluo-4, and Cameleon. The TIRF system is used in vesicular trafficking studies and also cell adhesion and migration experiments. The Andor iXon system is a high-speed EM CCD coupled with an Olympus microscope, designed to image fast calcium events in tissue preparations. For data deconvolution and analysis, we have two dedicated workstations running softWoRx (Applied Precision) for deconvolution, MetaMorph (Universal Imaging), Volocity (Perkin Elmer) and ImageJ (NIH) for general analysis and rendering. Here we review several ongoing research projects.

Supported by NIH Grant Numbers 5 P30 RR032135 from the COBRE Program of the National Center for Research Resources and 8 P30 GM 103498 from the National Institute of General Medical Sciences.

Poster #3

Constitutively Active TrkB Confers an Aggressive Transformed Phenotype to a Neural Crest Derived Cell Line

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Neuroblastoma arises from sympathoadrenal progenitors of the neural crest and expression of the neurotrophin receptor TrkB and its ligand, brain-derived neurotrophic factor (BDNF) is correlated with poor prognosis. Although activated TrkB signaling promotes a more aggressive phenotype in established neuroblastoma cell lines, whether TrkB signaling is sufficient to transform neural crest derived cells has not been investigated. To address the role of TrkB signaling in malignant transformation, we removed two immunoglobulin-like domains from the extracellular domain of the full length rat TrkB receptor to create a ΔIgTrkB that is constitutively active. In the pheochromocytoma- derived cell line PC12, ΔIgTrkB promotes differentiation by stimulating process outgrowth; however, in the rat neural crest derived cell line NCM-1, ΔIgTrkB signaling produces a markedly transformed phenotype characterized by increased proliferation, anchorage-independent cell growth, anoikis resistance, and matrix invasion. Furthermore, expression of ΔIgTrkB leads to up-regulation of many transcripts encoding cancer-associated genes including cyclind1, twist1, and hgf, as well as down-regulation of tumor suppressors such as pten, and rb1. In addition, ΔIgTrkB NCM-1 cells show a 21-fold increase in mRNA for MYCN, the most common genetic marker for a poor prognosis in neuroblastoma. When injected into NOD SCID mice, control GFP NCM-1 cells fail to grow while ΔIgTrkB NCM-1 cells form rapidly growing and invasive tumors necessitating euthanasia of all mice by 15 days post injection. In summary, these results indicate that activated TrkB signaling is sufficient to promote the formation of a highly malignant phenotype in neural crest derived cells.

Poster #4

TRPV4 Channels Tune Astrocyte Endfoot Ca2+ to Optimize Neurovascular Coupling

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A rise in intracellular calcium ($[Ca^{2+}]_i$) in astrocytes is required to communicate the energy demands of neurons to the cerebral microcirculation to precisely match local cerebral blood flow to neuronal metabolism. This “neurovascular coupling” (NVC) is essential for neuronal viability and homeostasis. A moderate rise in $[Ca^{2+}]_i$ in the perivascular “endfoot” terminals of astrocytes in response to neuronal activation produces vasodilation of the adjacent parenchymal arteriole, whereas high endfoot $[Ca^{2+}]_i$ can produce vasoconstriction. Thus, precise regulation of astrocytic $[Ca^{2+}]_i$ is necessary for proper function of the cerebral microcirculation. Transient receptor potential vanilloid 4 (TRPV4) channels are Ca^{2+}- permeant plasma membrane cation channels that participate in intracellular Ca^{2+} signaling in a variety of cell types. We previously reported that both the synthetic TRPV4 agonist, GSK1016790A, and the endogenous agonist, 11,12-EET, increase the frequency and amplitude of perivascular astrocytic endfoot Ca^{2+} oscillations in brain slices. Here we provide evidence from multiphoton confocal microscopy in brain slices and cerebral blood flow (CBF) measurement by laser Doppler flowmetry *in vivo*, that Ca^{2+} entry through TRPV4 in astrocytic endfeet contributes to NVC. Incubation of brain slices with the selective TRPV4 antagonist, HC-067047 (1 μM), attenuated the parenchymal arteriolar vasodilation to neuronal stimulation by 42% (n=5). This effect was accompanied by a reduction in pre-stimulation endfoot $[Ca^{2+}]_i$ and in the evoked increase in endfoot $[Ca^{2+}]_i$ in response to stimulation. Superfusion of HC-067047 over the somatosensory cortex in mice had no effect on resting CBF, but reduced the CBF response to contralateral whisker stimulation by 9% (n=7). These results indicate that TRPV4 channels tune perivascular astrocytic enfoot $[Ca^{2+}]_i$ to optimize NVC.

This study was supported by National Heart,Lung, and Blood Institute grants HL-44455, T32 HL-07944, and HL-095489.

Poster #5

Cavernous Nerve Dissection And Stretch Without Cutting Or Crushing Decreases Transcript Levels For The Neuronal Nicotinic Acetylcholine Receptor (nAchR) Subunit α3 and Postsynaptic Density (PSD)-93 in Male Mouse Major Pelvic Ganglia (MPG)

Beatrice M. Girard, John Tompkins, Margaret A. Vizzard, Rodney L. Parsons

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It is well known that transection or crush of autonomic postganglionic nerve axons causes synaptic depression. One contributing mechanism is a decreased expression of neuronal nAchRs and postsynaptic scaffolding protein PSD-93. We tested whether dissection of the cavernous nerve away from the prostate and urethra followed by a lateral stretch without cutting or crushing also causes a change in expression of transcripts for PSD-93 and the α3, β4 and α 7 subunits of the nAchR. Two groups of male mice underwent surgical procedures under anesthesia (1) axotomy group: unilateral section of the cavernous nerve and (2) dissect+stretch group: unilateral dissection of a short length of cavernous nerve away from underlying tissues followed by an ~5 mm lateral displacement without crushing or cutting the nerve. All animals recovered for 3 days post surgery before being euthanized and both MPG were harvested. QPCR was performed on extracts from the MPG and the contralateral un-operated MPG served as a control for the ipsilateral operated MPG. All transcript levels were normalized to transcript expression for the reference gene L32. Three days following surgery, transcript expression of α3 and PSD-93 were significantly reduced (α3: axotomy, $P< 0.003$; dissect+stretch, $P< 0.004$)(PSD-93: axotomy, $P< 0.02$; dissection+stretch, $P<0.03$) in the ipsilateral MPG compared to the contralateral MPG following axotomy or dissection/stretch. β4 transcript expression was reduced following both axotomy and dissection/stretch, but the difference was only significant for the axotomy group (axotomy: $P<0.05$; dissect+stretch, $P= 0.27$). α7 transcript expression was unchanged between the ipsilateral and contralateral MPG in either group. The MPG provide most of the innervation to the lower pelvic organs and during radical prostatectomy, axons derived from cells in the MPG are susceptible to injury resulting in unwanted side effects such as erectile dysfunction. Our results indicate that transcript expression for the nAchR α3 subunit and the scaffolding protein PSD-93 were reduced following axotomy or following cavernous nerve manipulation. Decreased nAchR α3 subunit expression would likely lead to a decrease in efficiency of synaptic transmission between parasympathetic preganglionic fibers in the pelvic nerve and postganglionic MPG cells that project to the penis through the cavernous nerve. An interruption of synaptic transmission could contribute to the loss of erectile function that occurs even with the nerve sparing surgical procedures.

Grants: RLP: COBRE P30, MV: DK051369, DK060481, DK065989

Poster #6

Plasticity in Transient Receptor Potential (TRP) Channel Expression in Urinary Bladder in Rodents with Urinary Bladder Dysfunction or During Postnatal Development

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TRP proteins associated with osmoregulation, thermal, chemical and mechanical signaling mechanisms are expressed within the lower urinary tract. Functional roles for TRP channels include visceral sensation including normal bladder function, bladder dysfunction and nociception. Transgenic mice (vasoactive intestinal polypeptide (VIP)$^{-/-}$ mice, nerve growth factor overexpressing (NGF-OE) mice) and rats with bladder inflammation induced by cyclophosphamide (CYP) exhibit bladder dysfunction. NGF exerts pro-inflammatory roles and roles in bladder overactivity. In contrast, VIP has anti-inflammatory roles in the urinary bladder. We hypothesized that bladder dysfunction involved a change in bladder TRPA1 and TRPV1-V4 expression. Furthermore, we hypothesized that postnatal maturation of bladder function involved altered bladder expression of TRP channels. We determined TRP channel bladder expression in NGF-OE, VIP$^{-/-}$, adult littermate wildtype (WT), early postnatal WT mice and rats with or without CYP-induced cystitis (4 hour (h), 48 h, chronic) using western blotting and Q-PCR. CYP-induced cystitis significantly ($p \leq 0.001$) increased TRPV1 protein expression in rat bladder with 4 h (2.2-fold) or 48 h (2.2-fold) cystitis in contrast to transient but significant ($p \leq 0.01$) decreases in TRPV1 mRNA with 4 h or 48 h cystitis. TRPV1 protein and mRNA bladder expression increased with development. TRPV1 mRNA was significantly ($p \leq 0.01$) reduced in VIP$^{-/-}$ and NGF-OE mouse bladder but protein expression was increased in VIP$^{-/-}$ mice. TRPV2 and TRPV3 mRNA were also significantly ($p \leq 0.01$) decreased in VIP$^{-/-}$ mice whereas TRPV4 mRNA was significantly increased in NGF-OE mice but was unchanged in VIP$^{-/-}$ mice. During postnatal development, TRPV4 mRNA was significantly increased in urothelium and detrusor, TRPV3 mRNA was significantly ($p \leq 0.01$) decreased in urothelium and TRPV2 mRNA was unchanged. TRPV4 protein was significantly ($p \leq 0.01$) increased in rat bladder with CYP-induced cystitis (4 h, 48 h, chronic). Cystitis-induced plasticity significantly ($p \leq 0.01$) increased TRPA1 mRNA expression in rat bladder with 48 h or chronic cystitis. TRPA1 mRNA expression was also significantly ($p \leq 0.01$) increased in NGF-OE but unchanged in VIP$^{-/-}$ mouse bladder. During postnatal rat development, TRPA1 mRNA was significantly ($p \leq 0.01$) increased in the urothelium but was unchanged in detrusor. These studies demonstrate plasticity of TRP channel expression in bladder with inflammation, during postnatal development or with NGF-OE or VIP deletion. Current studies are determining the potential functional effects of TRP channel plasticity on urinary bladder function.

NIH grants: DK051369, DK060481, DK065989, P30 RR032135, P30 GM103498

Poster #7

Co-Localization Of The Lynx1-A prototoxin and α7 Nicotinic Acetylcholine Receptors In Human Embryonic Kidney 293 Cells

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Lynx1-A is a Ly-6 family mammalian prototoxin, which is thought to act as a cholinergic brake by enhancing the rate of desensitization of the nicotinic acetylcholine receptor (nAChR) through its interactions at the binding pocket and has a particularly high affinity for the α7 nAChR homopentamer [2, 3, 4, 6]. While it has been suggested that Lynx1-A may have a modulatory role in nicotinic receptor trafficking and activity, subcellular co-localization has yet to be determined due to a lack of stable cell lines co-expressing Lynx1-A and the α7 nAChR and an absence of reliable antibodies against both proteins[2, 3, 5]. This project aimed to examine whether expression of Lynx1-A affects α7 nAChR subcellular localization, and to determine if and where co-localization occurs, since such sites may facilitate unique modulatory interactions. We hypothesized that Lynx1-A binds to the α7 subunit in the intracellular space and alters receptor trafficking to the plasma membrane. Human gene transcripts for Lynx1-A and α7 nAChR were ligated into plasmids with differing epitope tags, transformed into high efficiency *E. coli*, isolated and purified, then transfected into human embryonic kidney (HEK) 293 cells through calcium phosphate method. Intracellular co-localization was visualized through antibody staining against each epitope and immunofluorescence confocal microscopy. Though precise sites of subcellular co-localization could not be determined, substantial regions of overlapping expression lend support to the hypothesized modulatory interaction. Future works will include co-transfections with resistance to inhibitors of cholinesterase 3 (RIC3), a human protein that promotes α7 subunit trafficking, as well as assessments of receptor functionality, abundance and localization via α-BTX and nicotine binding experiments[1, 4].

Funded by: NIH Challenge Grant #1RC1DA028173

Poster #8

TRPC3 Channel Regulation of Sensory Afferent Extension in the Embryonic Avian Spinal Cord

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Development of dorsal root ganglia (DRG) sensory axons is characterized by a three step process whereby DRG axons grow towards and into the spinal cord at the dorsal root entry zone (DREZ), bifurcate and extend along the rostral-caudal axis and finally grow into the grey matter. Transient receptor potential channels (TRP) are a family of both selective and nonselective cation channels known to localize to developing growth cones and regulate Ca^{2+} influx. The TRP channel family is divided into seven subfamilies, including the canonical, Ca^{2+} permeable TRPC channels. The TRPC3/6/7 channel is activated intracellularly by PLCγ, an enzyme activated by Trk receptors. Activated PLCγ may cleave $PI_{4,5}P_2$ into DAG and IP3, which may then activate IP3 receptors on the endoplasmic reticulum allowing for release of intracellular Ca^{2+} stores. We have previously shown that inhibition of TrkB, its ligand Brain Derived Neurotrophic Factor (BDNF) or low extracellular Ca^{2+} results in significant inhibition of axon extension in the longitudinal pathway. Currently, our laboratory is interested in the potential roles Ca^{2+} and/or TRP channels may play in regulating the growth of sensory axons in the developing spinal cord. Using immunohistochemistry, we have shown TRPC3/6/7 immunoreactivity at stage 25 in development in both DRG and DREZ. Further, using an in vitro preparation we have shown that axon extension is significantly reduced by the application of Flufenamic, acid an inhibitor targeting TRP channels. Inhibitors of both PLCγ (u73122) and IP3 receptors, Xestospongin C also disrupt longitudinal extension of sensory afferents in the spinal cord. These data suggest a potential role for TRPC channels in sensory axon outgrowth at a time in development when axons are extending in the longitudinal pathway of the spinal cord. Supported by COBRE NIHRR16543.

Poster #9

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 demonstrated to play a role in stress, pain, and other emotion-related processes. An abundance of PACAP expressing fibers is found in the amygdala, a key site of integration for sensory and limbic signals involved in emotional regulation. Evidence suggests that PACAPergic fibers here may originate from neurons outside of the amygdala, however the identity of these neurons is currently unknown. The parabrachial nucleus (PBn) is known to relay signals involved in processing emotionally relevant pain stimuli and contains PACAP expressing cell bodies in the lateral PBn. This region has known axonal projections to the amygdala, some of which express calcitonin-gene related peptide (CGRP). These neurons are part of a spino-parabrachial-amygdaloid pathway that is implicated in the emotional responses to pain. We hypothesize that PACAP-containing fibers in the amygdala originate from cell bodies in the lateral PBn and are also part of this pathway. We first examined whether PACAP and CGRP are coexpressed in the amygdala and/or PBn using double-labeling immunohistochemistry. We are currently in the process of examining whether an anterograde neuronal tracer infused into the PBn localizes with PACAP-expressing fibers in the amygdala. Here, we present PACAP immunoreactivity in the amygdala and PBn and present preliminary evidence supporting PACAP coexpression with CGRP in the amygdala. These studies further substantiate a role for PACAP in the central integration of emotionally relevant sensory information.

Poster #10

Colocalization of the Prototoxin Prostate Stem Cell Antigen With α7 Nicotinic Acetylcholine Receptors and Acetylcholinesterase in Parasympathetic Neurons

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Prototoxins are novel endogenous 3-fingered proteins in the nervous system whose functions are not well understood. Our lab identified Prostate Stem Cell Antigen (PSCA), a prototoxin that is upregulated during development in the avian ciliary ganglion and decreases the responsiveness of α7 nicotinic acetylcholine receptors (nAChRs). The ciliary ganglion contains ciliary neurons and choroid neurons. This project was to determine if PSCA colocalizes with α7 nAChRs and acetylcholinesterase (AChE). To visualize PSCA, a V5-tagged PSCA was expressed in the retroviral vector RCASBP(A). Infected neurons were live-labeled on ice with anti-V5 antibody and either alexa-488 labeled αbtx or anti-acetylcholinesterase (1A2). Neurons were imaged using DeltaVision Deconvolution Restoration Microscopy. Deconvolved images analyzed using Vision software, ImageJ and Prism. Covariance of signal between V5 and either αbtx or acetylcholinesterase was measured using a Pearson's coefficient of colocalization. Results indicate PSCA colocalizes with α7 nAChRs in ciliary neurons, with a mean Pearson's coefficient of 0.5 $\pm$ 0.05 (SEM N=9), significantly higher ($p<0.0001$, Student's t-test) than the mean Pearson's of 0.2 $\pm$ 0.01 (SEM N=9) in choroid neurons. Although culturing neurons 24 hrs decreased colocalization, disrupting actin with latrunculin did not. Membrane bound AChE shows colocalization with α7 nAChRs in ciliary neurons but not choroid neurons (Pearson's of 0.06 $\pm$ 0.01 (SEM N=17)). Strong colocalization was observed between V5-PSCA and AChE (Pearson's 0.6 $\pm$ 0.04 (SEM N=8)). These results support a role for PSCA binding α7 nAChRs in pseudospines of ciliary neurons and suggests PSCA may be a novel binding partner of membrane-bound extracellular AChE.

Poster #11

Detection of L-Amino Acids Involves Taste Receptors in Addition to T1R1/T1R3

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Umami is a unique taste elicited by monosodium glutamate (MSG) and 5'-ribonucleotide monophosphates such as inosine monophosphate (IMP) and guanosine monophosphate (GMP). IMP and GMP are also known to synergize or potentiate (greater than an additive effect) the glutamate response. Research with L-glutamate, a prototypical L-amino acid that activates umami taste pathways, suggests a role of two likely G-protein coupled receptors, heteromers of taste receptor type 1, members 1 and 3 (T1R1/T1R3) and a taste variant of brain metabotropic glutamate receptor 4 (t-mGluR4). Molecular research using a HEK cell expression system has suggested that T1R1/T1R3 is a broadly tuned L-amino acid receptor (Nelson et al 2002). Further, Zhao et al. (2003) reported that behavioral preference and nerve responses to umami stimuli in T1R3 knock out (T1R3-/-) mice were totally abolished compared to control mice, arguing for the involvement of T1R1/T1R3 as the sole receptor in umami taste in mice. In contrast, Damak et al. (2003) found diminished but not complete loss of umami responses in T1R3-/- mouse, suggesting the involvement of multiple umami receptors. We are using a mouse model to study the transduction mechanism of L-amino acids. In this study we used calcium imaging of isolated taste sensory cells (TSCs) and taste buds to determine if : (1)TSCs are responsive to a panel of L-amino acids or only a particular subset of them (2)L-amino acids elicit synergy in TSCs when mixed with IMP (3)Receptors other than T1R1/T1R3 are also involved in L-amino acid

Poster #12

Intra-BNST Pituitary Adenylyl Cyclase Activating Polypeptide Increases Plasma Corticosterone

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In rats, exposure to repeated variate stress has been shown to increase PACAP and its cognate PAC1 receptor expression in the oval nucleus of the bed nucleus of the stria terminalis (BNST). BNST activity is critical for both anxiety-like behavior and physiological stress responses such as the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Moreover, changes in BNST PACAP signaling following repeated variate stress may be involved in the maladaptive changes in stress responding that may underlie anxiety disorders in humans. The current set of studies examined the dose-dependency and time-course of intra-BNST PACAP elevations in plasma corticosterone. Following intra-BNST PACAP38 infusion (0µg/µl, 0.1µg/µl, 0.5µg/µl or 1.0µg/µl in 0.05% bovine serum albumin vehicle), we observed a dose-dependent increase in plasma corticosterone levels 30 minutes following infusions. We further found that PACAP38 increased plasma corticosterone levels at both 30 minutes and 60 minutes, but not 4 hours or 24 hours following infusion. Importantly, the ability of intra-BNST PACAP38 infusion to increase plasma corticosterone was not due to spread of the drug into the nearby lateral ventricles as direct PACAP38 infusion into the lateral ventricles did not alter plasma corticosterone levels. These results suggest that intra-BNST PACAP can modulate corticosterone levels. The mechanism through which these effects may occur will be further discussed.

Poster #13

ATP Excites Mouse Vomeronasal Sensory Neurons through Activation of P2X Receptors

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Purinergic signaling through activation of P2X and P2Y receptors is critically important in the chemical senses. In the mouse main olfactory epithelium (MOE), adenosine 5'-triphosphate (ATP) elicits an increase in intracellular calcium ([Ca2+]I) and reduces the responsiveness of olfactory sensory neurons to odorants through activation of P2X and P2Y receptors. We investigated the role of purinergic signaling in vomeronasal sensory neuron (VSN)s from the mouse vomeronasal organ (VNO), an olfactory organ distinct from the MOE that responds to many conspecific chemical cues. Using a combination of calcium imaging and patch-clamp electrophysiology with isolated VSNs, we demonstrated that ATP elicits an increase in [Ca2+]I and an inward current with similar EC50s. Both types of responses elicited by ATP were not mimicked by adenosine, or the P2Y receptor ligands adenosine 5'-diphosphate, uridine 5'-triphosphate, and uridine-5'-disphosphate. Moreover, the increase in [Ca2+]I required the presence of extracellular calcium and the inward current elicited by ATP was partially blocked by the P2X receptor antagonists pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate and 2',3'-O-(2,4,6-trinitrophenyl) adenosine 5'-triphosphate. Consistent with the activation of P2X receptors, we detected gene expression of the P2X1 and 3 receptors in the VNO by RT-PCR. When co-delivered with dilute urine, a natural stimulus, ATP significantly increased the inward current above that elicited by dilute urine or ATP alone. Mechanical stimulation of the VNO induced the release of ATP, detected by luciferin-luciferase luminometry, and this release of ATP was completely abolished in the presence of the connexin/pannexin hemichannel blocker, carbenoxolone. We conclude that the release of ATP could occur during the activity of the vasomotor pump that facilitates the movement of chemicals into the VNO for detection by VSNs. This mechanism could lead to a global increase in excitability and the chemosensory response in VSNs through activation of P2X receptors. The presence of additional ATP release pathways and the role of the purinergic receptors in other aspects of VNO physiology are currently under investigation.

Poster #14

COBRE Neuroscience Cell and Molecular Biology Core Facility

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The COBRE Neuroscience Cell and Molecular Biology (CMB) Core at the University of Vermont was established to serve the neuroscience community by providing the equipment and training to incorporate cell and molecular approaches into their research. The core personnel include the core director, Dr. Sheryl White, and two full time technicians: Thomm Buttolph and Edward Zelazny. The CMB core provides one of the widest ranges of molecular biology services available in academic facilities in the country, including DNA services (construct design, cloning, PCR, site-directed mutagenesis and library construction), RNA services (Quantitative PCR, RNA isolation, RT-PCR, Northerns, RNase protection and differential display analysis), protein services (SELDI-TOF mass spectrometry biomarker profiling, protein extraction, SDS-PAGE, 2D-PAGE, western blotting and gel shift assays), cell culture services (primary/cell line culturing, transfection, reporter assays, immunohistochemistry, frozen/paraffin sectioning and slide staining), as well as specialized microscopy techniques (laser capture microdissection, Neurolucida morphometrics/stereology and cell counting). The equipment in the facility is available for researchers to use and includes specialized equipment such as ABI 7500FAST systems for qPCR, 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 Q24 Pyrosequencer, Biotek SynergyH4 plate reader, a Countess automated cell counter, an MP Bio FASTPREP 24 cell/tissue homogenizer, Qiagen's QIAcube and a Qiagility liquid handling system. Please visit us on the fourth floor of the Health Sciences Research Facility (HSRF 427) to see what equipment and services we can offer to advance your research.

Poster #15

Executive Function Effects of Voluntary Exercise and Methylphenidate in Developing Male Rats

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Voluntary exercise may produce some of its behavioral benefits by acting on dopamine and norepinephrine systems. Methylphenidate (MPH) is a commonly prescribed dopamine and norepinephrine reuptake inhibitor used to treat attention-deficit/hyperactivity disorder in children and adolescents. In two experiments, we gave adolescent (30-44 days old) male rats either free access to a running wheel in their home cage or a daily injection of 2 mg/kg MPH. A third group received neither. In Experiment 1, rats underwent maze testing as young adults (60 days old; i.e., 2 weeks after treatment ended). In Experiment 2, rats underwent maze testing as adolescents (45 days old; i.e., immediately after treatment). Maze testing involved training rats to discriminate rewarded from unrewarded arms in a T maze based on one stimulus dimension of the arms (e.g., black vs. white), and then to set-shift, in which they have to discriminate based on another stimulus dimension of the arms (e.g., smooth vs. rough). In Experiment 1 (delayed testing), preliminary data suggest that MPH, but not exercise, improved set-shifting. In Experiment 2 (immediate testing), preliminary data suggest that exercise, but not MPH, improved set-shifting. Exercise may need to be continuous in order to benefit executive function.

Poster #16

The Effects of Secretin Infusions into Different Areas of Cerebellar Cortex on Rat Eyeblink Conditioning

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Eyeblink conditioning (EBC) is a well-studied form of classical conditioning supported by plasticity in the cerebellum. EBC involves trials in which a tone conditioned stimulus (CS) precedes an eyelid stimulation unconditioned stimulus (US). The conditioned response (CR) is an eyeblink to the CS. Both Purkinje cells (PCs) in cerebellar cortex and interpositus nucleus (IPN) neurons receive CS and US inputs. PCs normally inhibit the IPN but lift this inhibition during EBC. PCs are powerfully regulated by basket cells (BC), a cerebellar inhibitory interneuron whose axon terminals have the highest concentration in the brain of the voltage-gated K^+ channel α-subunit, Kv1.2. Previous research shows that PCs express and release secretin, surface Kv1.2 in BC terminals is reduced by secretin and secretin increases IPSCs in PCs. We have shown that infusing secretin into lobulus simplex in cerebellar cortex facilitates conditioning presumably by increasing inhibition of Cs, thereby reducing inhibition of IPN neurons. In previous research relatively large infusions were made into the cerebellar cortex to facilitate conditioning. The current experiment seeks to determine a localized area within the cerebellar cortex that may control acquisition of EBC. Four groups of rats received small infusions of either secretin or vehicle into the lobulus simplex or anterior lobe of the cerebellar cortex prior to EBC. Results indicated no differences in percentage of CRs between vehicle and secretin infused rats in either location. We are currently examining a slightly more lateral location in cerebellar cortex as a locus for the facilitating effects of secretin on EBC.

Poster #17

Exercise and Subchronic Fluoxetine Produce a Reduction in Anxiety in C57 Mice That Is Greater Than Exercise or Fluoxetine Alone

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We have shown that voluntary exercise in mice is associated with reduced anxiety across several anxiety models. We and others have shown that exercise may exert its anxiolytic effects by altering the responsiveness of central serotonin systems. Because chronic administration of drugs that modulate serotonin systems (e.g., SSRI's) can also reduce anxiety, there may be overlap between the mechanisms underlying the anxiolytic effects of exercise and chronic SSRI's. In order to determine whether the anxiolytic effect of voluntary exercise summates with the anxiolytic effect of subchronic fluoxetine, Male C57BL/6J mice were given either functioning or non-functioning (i.e., locked) running wheels, and after 14 days of wheel access, exercise and Sedentary groups were given 14 days of once daily injections of a moderate dose of fluoxetine (10 mg/kg) or vehicle. The anxiolytic effects of exercise, fluoxetine and the combination of exercise and fluoxetine were assessed using stress-induced hyperthermia and a challenge with the anxiogenic drug metachlorophenylpiperazine (mCPP). Subchronic fluoxetine alone did not reduce stress-induced hyperthermia nor did it attenuate the anxiogenic effect of mCPP. Consistent with previous data from our lab, exercise alone reduced stress-induced hyperthermia and attenuated the anxiogenic effect of mCPP. Importantly, the combination of exercise and fluoxetine resulted in a greater reduction in stress-induced hyperthermia and the anxiogenic effect of mCPP than exercise alone. These data suggest that the anxiolytic effects of exercise can be boosted with a moderate dose of fluoxetine that itself does not produce a reduction in anxiety.

Poster #18

Different Subregional Effects Of Intra-BNST PACAP on Stress And Anxiety-Like Behavior

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We have previously shown that pituitary adenylate cyclase-activating peptide (PACAP) signaling in the bed nucleus of the stria terminalis (BNST) mediates many behavioral consequences of repeated/chronic stressor exposure. PACAP is elevated in the BNST oval nucleus following repeated variate stress, BNST PACAP antagonism attenuates many of the consequences of repeated variate stress, and BNST PACAP38 (PACAP agonist) infusion increases anxiety-like behavior and causes dose-dependent anorexia and accompanying weight loss measured 24 hours later. We typically aimed our infusion at the region around the BNST oval nucleus; however, histological analyses of our prior studies revealed that posterior BNST infusions targeting the BNST principal nucleus were often more effective in producing anorexia and weight loss than anterior infusions targeting the BNST oval nucleus. Hence, we bilaterally infused rats with 1.0µg/0.25µL PACAP38 or a vehicle into either the BNST oval nucleus or BNST principal nucleus and assessed weight loss or anxiety-like behavior on the elevated plus maze (EPM) in separate experiments. In rats not exposed to the EPM, anorexia and weight loss was observed following PACAP infusion into the BNST principal nucleus, but not following PACAP infusion into the BNST oval nucleus. Anxiety-like behavior on the EPM was not elevated by small volume infusions of PACAP into either BNST subregion; however, we found that the combination of EPM-exposure and PACAP infusion into the BNST oval nucleus produced weight loss. These results indicate that the PACAP infusion within the BNST oval nucleus might synergize with anxiogenic/stressful stimuli to produce anorexia and accompanying weight loss.

Poster #19

Working Memory Ability Modulated Brain Functional Connectivity during Dopamine Agonist Stimulation in Postmenopausal Women

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Declines in dopaminergic functioning may be responsible for cognitive changes after menopause. To date, no study has examined baseline working memory influences on resting state functional connectivity during dopaminergic agonist drug administration. This would allow for an examination of the dopaminergic contribution to brain functional connectivity in networks responsible for cognition that may decline as a result of the hormonal change at menopause. Eighteen healthy, cognitively normal, postmenopausal women underwent a resting state scan during fMRI on two study days where women received randomly and blindly 1.5 mg orally of the dopaminergic agonist bromocriptine or matching oral placebo. Women also performed the Letter-Number sequencing test to asses working memory ability and a median split was performed to separate women with high and low working memory ability. We examined resting state functional connectivity during fMRI after dopaminergic drug challenge. Increased functional connectivity for women with low working memory ability was found for bromocriptine compared to placebo in the left and right inferior parietal lobule (BA 39) and the left middle frontal gyrus (BA 8). For the women with high working memory ability, bromocriptine increased functional connectivity compared to placebo in the medial frontal gyrus (BA 11). Overall, dopaminergic agonist challenge resulted in increased functional connectivity in a frontal-parietal network associated with working memory performance in postmenopausal women with low working memory ability. This relationship between dopaminergic system functioning and brain connectivity patterns implies that the inverted-U shaped relationship between dopaminergic systems and brain functioning is intact after menopause.

Poster #20

Adults with ADHD Show Reduced Prefrontal Cortex and Subcortical Activation in a fMRI Delay Discounting Task

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In the United States 9.5% of all children (4-17 years) have ever been diagnosed with attention deficit-hyperactivity disorder (ADHD). In many persons, the symptoms of ADHD persist into adulthood. An important characteristic of childhood ADHD is a bias towards immediate rewards. To assess the persistence of this bias into adulthood and to determine its neurobiological basis, a hypothetical delay-discounting task was administered in conjunction with functional magnetic resonance imaging. Study participants included adults who met DSM-IV criteria for combined type ADHD as well as age and sex matched controls. Subjects were asked to choose between a small immediate reward and a larger delayed reward. With each subsequent choice, the parameters of the question were adjusted with an individually-tailored adaptive algorithm to converge on each participant's indifference point, the point at which they give equal weighting to immediate and delayed rewards. Robust task activation was observed in the ventral striatum and prefrontal cortex. Additionally, there was significantly reduced BOLD signal in regions associated with the default mode network. Adults with ADHD discounted delayed rewards more steeply and showed less task activation in cortical and subcortical regions than controls. More specifically, subjects with ADHD showed significantly lower activity than controls in the cingulate cortex and ventral striatum as well as less deactivation of the default mode network during task choices. Additionally, controls showed greater left dorsolateral prefrontal cortex activation when selecting the later reward than ADHD subjects.

Poster #21

Burn and Earn: A Randomized-Controlled Trial Examining the Efficacy of Incentives to Motivate Fitness-Center Attendance in College Freshmen

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Objective: This study determined whether exercise habits established with the provision of weekly incentives would persist after the discontinuation, or decreased frequency, of incentives. The study also examined weight change of first-year students.

Methods: Randomized-controlled trial with control, discontinued-incentive, and continued-incentive conditions involving 117 first-year students. For 12 weeks in fall semester 2011 students in both incentive conditions received monetary payments for meeting fitness-center use goals. For 12 weeks in spring semester students in the discontinued-incentive group no longer received incentives to meet goals, and participants in the continued-incentive group received payments on a variable-interval schedule. Electronic ID-card check-in and check-out records were used to track fitness-center attendance.

Results: When incentives were discontinued, fitness-center goal achievement decreased from 63% of goals met with weekly incentives to 3% of goals met in the discontinued-incentive group, equivalent to the level of goal completion in the control group. When rewarded on a variable-interval schedule, those in the continued-incentive group met the goals 39% of the time, which was a significantly higher goal completion rate than the discontinued-incentive and control groups, $\chi 2=(2, n=113)=21.07, p<0.001$. A mixed-model analysis revealed that there was not a significant interaction between condition and BMI change, $F(6, 332)=0.67, p=0.68$, nor was there a significant change in BMI over time, $F(2, 217)=0.56, p=0.58$.

Conclusions: When incentives were discontinued, students no longer met fitness-center attendance goals. Attendance was maintained when rewards were provided on a variable-interval schedule. Although all groups experienced some weight gain, weight gain was not significant.

Poster #22

Characterizing PACAP Receptors Involved In Anxiety-Like Responses: Evidence for HPA-Axis Activation by BNST PACAP and PAC1 Receptor Signaling

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Exposure to repeated or chronic stress has been shown to dysregulate normal stress circuits, such as the hypothalamic-pituitary-adrenal (HPA) axis, and lead to maladaptive physiological and behavioral responses that can manifest as anxiety disorders. These maladaptive responses are thought to be mediated by plasticity within brain regions that regulate the emotional and neuroendocrine responses to stressors. One such area, the bed nucleus of the stria terminalis (BNST), has been shown to undergo neural plasticity in response to exposure to stressors, participate in anxiety-like behaviors, and alter HPA-axis functioning. Our lab has previously reported that in a rodent model, repeated variate stress increases pituitary adenylate cyclase activating polypeptide (PACAP) and PAC1 receptor transcript expression selectively within the dorsal BNST. In addition, a single injection of PACAP into the BNST mimics many of the consequences of repeated stressor exposure, including increased anxiety-like behavior, decreased food intake and weight loss, and increased circulating stress hormone levels. As PACAP binds PAC1 and VPAC receptors, we sought to elucidate the specific receptor subtype involved in the PACAP-mediated anxiety-like responses. We have now compared the effects of intra-BNST infusion of the specific PAC1 receptor agonist, maxadilan, with those after activation of VPAC receptors by VIP. Results suggest that PAC1 receptor activation within the BNST is necessary and sufficient to produce weight loss, anorexia, and anxiety-related behaviors. We also further characterized the neuroendocrine stress responses along the HPA-axis following acute infusion of PACAP into the BNST. Hence BNST PACAP and PAC1 receptor signaling appear central in coordinating the emotional and neuroendocrine responses to chronic stress.

Poster #23

Regulation of Impulsive Circuitry: Function of Emotional Faces

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

Poster #24

Cerebellar Contributions to Impulsivity Phenotypes

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Impulsivity is a central clinical feature of many psychiatric illnesses including Attention Deficit/Hyperactivity Disorder (ADHD) and substance use disorders. It has long been known that cerebellar abnormatlities, both structural and functional are associated with ADHD, however how these abnormalities contribute to specific symptoms is unknown. While the role of the cerebellum in motor function and dysfunction has been well characterized, increasing evidence suggests that the cerebellum is important in many non-motor higher level cognitive processes as well. For example, it has been hypothesized that cerebellar dysfunction in ADHD may directly impact basic cognitive processes that give rise to symptoms in ADHD including impulsivity. Therefore, this study tested the hypothesis that anatomic differences in cerebellar volume are differentially associated with different forms of impulsivity

19 (89% female) young adults (age 19-22) participated in this study. Subjects completed two tests to characterize different forms of impulsivity; the Stop Signal Task and the Delay Discounting task, and completed an MRI brain imaging anatomical scan. Subjects were characterized (using a median split) as high or low impulsive on each type of impulsivity. Volumetric analysis of the cerebellum was completed to determine differences in cerebellar volume associated with each type of impulsivity. Differences were found based on type of impulsivity (response inhibition compared to delay discounting) supporting both a role for cerebellar dysfunction associated with impulsivity, and supporting dissociable contributions of cerebellar circuitry to different forms of impulsivity.

Poster #25

Neurobiological Sex Differences in Adolescent Drinkers

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Understanding the neurobiological effects of alcohol use in adolescents is of vital importance. Nearly 40% of individuals who have exposure to alcohol before the age of 14 exhibit heightened levels of alcohol abuse and dependence. Alcohol use is associated with maladaptive functioning in frontal and subcortical areas known to be involved in reward. Few neuroimaging studies have investigated sex differences in the neurobiology of reward; fewer have explored these differences in adolescent drinkers.

Data were acquired from 1313 (706 female) 14-year-old adolescents tested as part of the multi-site IMAGEN project. Alcohol use was determined with The School Survey Project on Alcohol and Drugs (ESPAD). The Monetary Incentive Delay (MID) task was used to assess reward processing during functional magnetic resonance imaging (fMRI). Additionally, Voxel Based Morphometry (VBM) was utilized to explore brain volume effects of sex and alcohol use.

In a number of fronto-parietal cortical and midbrain regions, males showed heightened activation during MID reward outcome and anticipation compared to females. During reward outcome male drinkers demonstrated heightened activation compared to male non-drinkers. Female drinkers had reduced activation compared to non-drinking females. Drinking in females demonstrated a robust volume reduction in the left cerebellum compared to non-drinking females which was not seen with either drinking or non-drinking males.

These analyses suggest subcortical reinforcement systems are reduced in female drinkers compared to female non-drinkers and heightened in males drinkers compared to male non-drinkers. Compromise in these systems may confer risk for initial use and subsequent alcohol use disorders in male and female adults.

Poster #26

Changes In White Matter As A Measure Of Neuroplasticity Following Cognitive Behavioral Therapy For Coping With Chronic Pain

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Cognitive Behavioral Therapy (CBT) has the ability to alter the ways in which we think, feel, behave, and perceive pain. Using functional Magnetic Resonance Imaging (fMRI), our lab has previously demonstrated that there are differences in neural function between the brains of healthy volunteers and chronic pain patients, and that these functional differences can be attenuated after as few as three months of CBT for coping with chronic pain. Further, gray matter volume significantly increases within functionally relevant brain regions after completion of CBT (in preparation for publication). We hypothesize that these changes in gray matter structure and function are accompanied by corresponding neuroplastic changes in the properties of white matter, as measured by Diffusion Tensor Imaging (DTI). Specifically, we expect to observe changes in fractional anisotropy (FA) within tracts that connect brain regions associated with perception of pain and that show structural and functional differences after CBT. We predict that these changes will also correlate with improvements in clinical outcomes.

Poster #27

Anatomical Changes Following Cognitive Behavioral Therapy in Patients with Chronic Musculoskeletal Pain

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Several studies have reported functional and anatomical changes related to chronic pain conditions. However, there is limited research examining the plasticity of the human cortex in response to therapeutic interventions. The aim of this investigation was to demonstrate the reversibility of anatomical decrements in patients with chronic musculoskeletal pain after a psychotherapeutic intervention. Anatomical neuroimaging was performed in a sample of patients with chronic musculoskeletal pain before and after 11-week cognitive behavioral therapy (CBT) and compared to a control group of patients receiving educational materials. Structural volumetric measures were correlated with clinical outcomes. The course of CBT led to significantly more improvement in clinical measures. Whole brain analysis revealed a significant cluster in the secondary somatosensory area (S2). S2 gray matter volume (GMV) went down after therapy in the CBT group compared to the educational materials group. Decrease in the S2 GMV after CBT correlated better with improvements in the sensory rather than affective aspects of the pain experience and could reflect better coping with afferent noxious inputs. These results add to mounting evidence that CBT can be a valuable treatment option for chronic musculoskeletal pain, and that treating pain can partially reverse previously reported abnormal brain anatomy associated with chronic pain.

Poster #28

Estradiol Treatment in Postmenopausal Women Increased Functional Connectivity in Brain Networks Associated With Cognition

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The decrease in circulating estrogens in women after menopause has direct effects on cognitive functioning. Estrogen treatment in postmenopausal women influences frontal lobe and hippocampal functioning and improves performance on working memory and episodic memory tasks. However, the brain mechanisms involved have yet to be elucidated. Thus far no study has examined the effects of estrogen treatment in postmenopausal women on functional connectivity of large scale brain networks involved in cognition in an effort to understand hormone influences on the functional organization of the brain. Twenty-four healthy, cognitively normal postmenopausal women were randomly assigned to either three months of oral 17-beta estradiol or matching placebo. At baseline and after three months of treatment women took part in fMRI scanning sessions to examine estrogen effects on functional connectivity. Results showed that the estrogen treated group had increased connectivity between hippocampal, posterior cingulate, inferior parietal, and insula regions compared to the placebo treated group. These results demonstrate the ability of estradiol treatment after menopause to modulate brain functional connectivity. The increase in connectivity may be one mechanism underlying how estrogen benefits cognition supported by these functional networks in postmenopausal women.

Poster #29

5HT4 Receptors in the Colon and Duodenum Mucosa Educes 5-HT release

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Introduction. Serotonin (5-HT) is a signaling molecule that is abundant in the digestive tract.[1] 5-HT is synthesized and stored by enterochromaffin (EC) cells, which release 5-HT in response to pressure and chemical stimuli. When released, 5-HT activates enteric reflexes that promote motility and fluid secretion. Recently, we discovered that 5-HT4 receptors (5-HT4R), which are the therapeutic targets of agonists used to treat constipation, are expressed by epithelial cells, including EC cells, in the intestines (Hoffman et al, Gastroenterology, 2012, 142:844-854). In the current study, we tested the hypotheses that (1) components of the intestinal lumen during digestion can stimulate 5-HT release, and (2) 5-HT4R agonists will activate larger 5-HT release events in the colon than in the duodenum because the receptor is expressed at the highest level in the colon.

Methods. Serotonin release was evaluated by *in vitro* amperometry with diamond microelectrodes in mouse and guinea pig specimen. 5-HT4R compounds included the agonist, cisapride and the antagonist, GR113808.

Results. 5-HT release was stimulated by HCl (pH, 2.6), sodium deoxycholate (4 mM), and cisapride (1µM) in both mouse and guinea pig, and both in the duodenum and colon. Administration of the 5-HT4R agonist educed 5-HT release from EC cells. There was a higher guinea pig basal release in the duodenum than the colon; consistent with the 5-HT gradient that exists between these two organs. The exploration did not find a difference between serotonin release from EC cells in mice and guinea pig tissue indicating they are comparable animals.

Conclusions. EC cells in the small and large intestines respond to similar secretagogues. Higher levels of 5-HT release are detected in the duodenum, which contains the highest density of EC cells, and the highest 5-HT content throughout the GI tract.

Poster #30

The Neurobiology of Successful Heroin Abstinence

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Disruption of the brain's executive functioning, including a hindrance of prefrontal and subcortical processes regulating inhibition, often characterizes substance abusers. Such individuals exhibit a propensity to choose immediate rewards with disregard for a more valuable long-term goal. However, the literature on the neurobiology of successful abstinence is scarce. We examined the neurobiological basis of successful abstinence by implementing a delayed discounting task (which assesses the extent to which rewards are devalued as a function of how long one must wait for them) in concurrence with fMRI. Behavioral and functional data were collected from a population of former heroin users including a methadone-maintained group and an abstinent group, and a control group matched for age and gender. To measure the rate of temporal discounting, subjects were asked to choose between smaller sooner rewards and larger later rewards. There was no difference in discounting rates between the two groups of former heroin users, but both groups discounted significantly more than the control group. The task produced robust activation in the ventral striatum and prefrontal cortex, as well as reduced BOLD signal in the default mode network. Clinical groups showed heightened activity in cognitive control regions but showed different activation patterns. Relative to healthy controls, the methadone-maintained group showed elevated levels of medial prefrontal activity, a brain region thought to be involved in suppressing the desire for immediate rewards. The abstinent group showed greater activity in the anterior cingulate, a region associated with behavior monitoring. These findings support the notion that successful abstinence is characterized by intensified executive functioning with different processes being involved at different stages of abstinence.

Poster #31

Natural Variation in the Murine Y Chromosome Influences Gene Regulation And Susceptibility to Experimental Allergic Encephalomyelitis

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A full appreciation for the architecture of the regulatory genome is critical to delineate the underlying biological components controlling susceptibility to complex diseases. Mammalian sex chromosomes influence gene regulation, but whether the X chromosome primarily mediates these effects, or whether the Y chromosome (ChrY) also possesses regulatory properties, remained unclear. Using ChrY consomic strains of mice, we show that susceptibility to experimental allergic encephalomyelitis (EAE) is controlled by natural variation in ChrY, which possesses genome-wide regulatory properties that influence the activation and signaling profiles of immune cells linked to disease. In an SJL-ChrY consomic strain, genetic variation in ChrY results in altered expression of molecules within the IL-10 signaling pathway, which contributes to the activation and maturation deficiency observed for SJL macrophages. In a B6-ChrY consomic strain, ChrY-mediated changes in the $CD4^+$ T cell transcriptome translates directly into altered protein synthesis as a result of biological changes in T cell activation and signaling. ChrY-linked regulatory variation in chromatin remodeling gene expression and disease susceptibility correlates with natural variation in the copy number of ChrY multicopy genes among mouse subspecies. Thus, the ChrY-mediated regulatory variation in genome-wide gene expression establishes this chromosome as an expression quantitative trait locus in EAE and as a critical component of the regulatory genome in males.

Poster #32

An Enhanced Myogenic Vasodilatory Response to Hypotension in Posterior Cerebral Arteries of Pregnant Rats is Nitric Oxide Dependent

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Introduction: Cerebral blood flow autoregulation (CBFAR) functions to maintain constant blood supply to the brain despite fluctuations in blood pressure (BP). The myogenic vasodilation in response to hypotension is a critical component of CBFAR. In the present study, we investigated the influence of pregnancy on myogenic vasodilation and the autoregulatory response to hypotension and hemorrhage.

Methods: Posterior cerebral arteries (PCAs) from nonpregnant (NP) and late-pregnant (LP, E20) SD rats were dissected and cannulated in an arteriograph chamber. Myogenic vasodilation was measured in the absence (n=10/group) or presence of the NOS inhibitor N^{ω} –nitro-L-arginine (L-NNA, 0.1mmol, n=7/group) by decreasing pressure from 125 to 5 mmHg and recording luminal diameter. Autoregulation in response to hemorrhagic hypotension was measured *in-vivo* in NP and LP rats (n=8/group) anesthetized with chloral hydrate and ventilated to maintain blood gases. CBF in the PCA territory was measured using laser Doppler.

Results: PCAs from NP and LP rats developed similar myogenic tone at 125 mmHg (33 % ± 3 and 34 % ± 2; ns). When pressure was decreased, PCAs from LP animals dilated becoming significantly larger at 50 mmHg vs. starting diameter at 125 mmHg (183 µm ± 8 vs. 147 µm ± 5; $p<0.05$). The dilation of PCAs from LP animals was prevented by L-NNA treatment (not shown). In contrast, PCAs from NP animals dilated less (161 µm ± 11 at 50 mmHg vs. 145 µm ± 9 at 125 mmHg; ns), and L-NNA treatment did not affect myogenic vasodilation. *In-vivo* when BP was lowered by hemorrhage the change in CBF became significant vs. baseline at 90 mmHg in NP animals; however, BP decreased to 60 mmHg before CBF was different vs. baseline in LP animals, suggesting CBFAR to decreased pressure is more stable during pregnancy.

*Conclusion***:** These results suggest there is an enhanced myogenic vasodilatory response to decreased intraluminal pressure in the pregnant state that is due to NO. This potential role for NO in pregnancy may promote greater effectiveness of CBFAR during hypotension and hemorrhage.

Key Words: Maternal Brain, Hypotension, Myogenic Vasodilation

Poster #33

Profound Decrease in Myogenic Tone of Parenchymal Arterioles in a Genetic Model of Cerebral Ischemic Small Vessel Disease

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Dominant mutations in NOTCH3 gene induce the most common heritable cause of stroke and vascular dementia, named Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL). Recently, a mouse genetic model for CADASIL was developed, exhibiting the main features of the disease. Importantly this model established that cerebrovascular dysfunction is an early event that precedes white matter lesions (Joutel et al., JCI 2010). Here we report the effects of CADASIL on arterioles within the brain. Elevation of intravascular pressure to 20 mm Hg constricted isolated parenchymal arterioles (PAs) from WT and CADASIL brains to the same extent (34%). However, above 30 mm Hg, CADASIL PAs lost myogenic tone. At 40 mm Hg, PAs from WT and CADASIL constricted 39% and 25%, respectively, and the membrane potential of the CADASIL arterioles was 10 mV more hyperpolarized. Consistent with these finding, the current density of voltage-dependent K+ channel currents was 30% greater in myocytes from CADASIL vs WT. These results indicate that the CADASIL mutation has a profound effect on PAs tone and point to a critical role for Kv channels. The present work provides new insights about the earliest events that initiate brain lesions in this model of cerebral ischemic small vessel disease.. Supported by AHA 09POST2290090 (FD), the NIH R37DK053832, PO1HL095488, RO1HL44455, RO1HL58231, the Totman Trust for Medical Research and the Fondation Leducq for the Transatlantic Network of Excellence on the Pathogenesis of Small Vessel Disease of the Brain.

Poster #34

Characterization of Transforming Growth Factor Beta 1, 2 and 3 (TGF-β1, TGF-β2 and TGF-β3) and receptors in rat urinary bladder following cyclophosphamide (CYP)-induced cystitis

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Diverse proinflammatory cytokines have been identified in micturition reflex pathways and are recognized to contribute to urinary bladder dysfunction following CYP-induced cystitis. The pleiotropic cytokines, TGF-β (1, 2 and 3), are proposed to act as cellular switches that regulate immune function, proliferation, and epithelial-mesenchymal transition. TGF-β (1, 2 and 3) and receptors were examined in urinary bladder of control rats and those treated with CYP of varying duration (4 hour (hr), 48 hr or chronic). Transcript and protein expression of TGF-β (1, 2 and 3) and receptors were studied using qRT-PCR, ELISAs and immunohistochemistry with image analyses. TGF-β1 mRNA expression significantly increased in the urothelium or detrusor 48 hr after CYP-induced cystitis. TGF-β2 and TGF-β3 mRNA expression significantly increased in the urothelium with 48 hr or chronic CYP treatments. TGF-receptor (R) 1, TGF-R2 and TGF-R3 mRNA significantly increased in the urothelium with 48 hr or chronic CYP treatment whereas TGF-R1, TGF-R2 and TGF-R3 expression increased in detrusor only with chronic CYP treatment. TGF-β1 and TGF-β3 protein expression significantly increased in the urinary bladder with 48 hr CYP treatment whereas TGF-β2 protein expression exhibited a transient decrease in bladder with 4 hr CYP treatment. TGF-β1- and TGF-R1-immunoreactivity (IR) significantly increased above threshold in the urothelium with 48 hr and chronic CYP treatment. The current studies demonstrate expression and plasticity of TGF-β (1, 2 and 3) and associated receptors in urinary bladder following CYP-induced cystitis. Future studies will evaluate the role of TGF-β/receptor signaling in urinary bladder function following CYP-induced cystitis.

Disclosures: E. Gonzalez: None. B.M. Girard: None. S. Malley: None. M.A. Vizzard: Research Grant; DK051369, DK060481, DK065989, 5 P30 RR 032135, 8 P30 GM 103498.

Support: NIH Grant DK051369, NIH Grant DK060481, NIH Grant DK065989, National Center for Research Resources 5 P30 RR 032135, National Institute of General Medical Sciences 8 P30 GM 103498

Poster #35

The Effect of Cyclophosphamide on c-Fos-like labeling in the Nucleus of the Solitary Tract

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Cyclophosphamide is a chemotherapeutic agent used for the treatment of lymphoma, leukemia and other cancers. It is a pro-drug, a chemical that only becomes biologically active in the body. Cyclophosphamide is activated when metabolized by the liver to create alkylating agents that bind to, and damage, DNA. The damage arrests the cell cycle until the DNA is repaired, but it is often so extreme that cells are no longer viable and go through apoptosis or necrosis. Unfortunately, due to the lack of specificity, non-cancerous, highly dividing cells are often damaged as well. The lingual epithelium is one such highly dividing cell population that can be damaged and can be extensive enough to result in taste deficits. To test the hypothesis that the peripheral damage of cyclophosphamide affects the signaling of taste information in the central nervous system, mice were injected with cyclophosphamide and allowed to drink solutions containing taste stimuli at different time points post-injection. In healthy mice, taste stimulation results in c-Fos protein expression in activated neurons in the nucleus of the solitary tract (NST). Using immunohistochemistry targeted towards c-Fos-like proteins, it was found that fewer neurons in the NST were c-Fos-like immunopositive in mice injected with cyclophosphamide over controls for at least 10 days after drug administration. This indicates that a single dose of cyclophosphamide can disrupt taste function for a long period, which can negatively impact patient nutrition and quality of life.

Poster #36

Low-Level Methylmercury Enhances CNTF-evoked STAT3 Signaling and Glial Differentiation in Cortical Progenitor Cells

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Sub-cytotoxic levels of mercury compounds may affect mechanisms essential for the proper development of the fetal nervous system. The present study investigates whether low doses of methylmercury (MeHg) and mercury chloride (HgCl2) can modulate the activity of JAK/STAT signaling, a pathway that promotes gliogenesis. Here we report that acute sub-cytotoxic doses of MeHg enhance ciliary neurotrophic factor (CNTF) evoked STAT3 Tyrosine-705 phosphorylation in SH-SY5Y and mouse cortical neural precursor cells (155% at 1000 nM in SH-SY5Y; $p<0.05$, and ~170% at 30 and 300 nM in NPCs; $p<0.05$; ANOVA w/ Bonferroni tests). This effect is specific for MeHg, since HgCl2 fails to enhance JAK/STAT signaling in NPCs and significantly inhibits STAT3 phosphorylation in SH-SY5Y (20% of control at 100uM $p<0.05$). In NPCs the effects of low dose MeHg are followed by increases in the expression of the STAT3-target genes glial fibrillary acidic polypeptide (GFAP) and Suppressors of Cytokine Signaling (SOCS3) at 12 h as determined by quantitative real-time PCR (GFAP: 1.66-1.59 fold increase at 30 and 300nM, respectively; $p<0.05$. SOCS3: 1.91-2.06 fold increase at 30 and 300nM, respectively, $p<0.001$) and an increase in the proportion of cells expressing GFAP following two days differentiation in the presence of CNTF as shown by immunocytochemistry (189% increase in GFAP+ cells relative to CNTF/vehicle control at 300nM; $p<0.05$) and confirmed with Western Blotting. Higher, near-cytotoxic concentrations of MeHg inhibit JAK/STAT and lead to increased production of superoxide as shown by nitroblue tetrazolium assay. Lower concentrations of MeHg effective in enhancing JAK/STAT signaling do not result in a detectable increase in NBT signal. These findings suggest that low concentrations of MeHg, but not HgCl2, may inappropriately enhance a glial differentiation, and that the mechanism causing this enhancement is distinct from the ROS-associated cell death observed at higher concentrations of MeHg and HgCl2.

Poster #37

Inversion of Neurovascular Coupling after Subarachnoid Hemorrhage

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Aneurysmal subarachnoid hemorrhage (SAH) is associated with high rates of morbidity and mortality. The cellular events contributing to SAH-induced ischemic neuronal damage, a major cause of poor outcome, are still elusive. Here we examined the impact of SAH on neurovascular coupling (NVC) in brain slices from control and SAH model rats. Brain slices were loaded with the fluorescent Ca^{2+} indicator dye, fluo-4, and astroctyic endfoot Ca^{2+} concentration and adjoining parenchymal arteriolar diameter were simultaneously measured using two-photon and infrared-differential interference contrast (IR-DIC) microscopy. As anticipated, neuronal activation by electrical field stimulation (EFS) caused an increase in endfoot Ca^{2+} followed by arteriolar dilation in brain slices from control rats. Remarkably, EFS caused a similar increase in astrocyte endfeet Ca^{2+} but induced vasoconstriction rather than vasodilation in brain slices from day 4 SAH animals. Similarly, Ca^{2+} uncaging in astrocyte endfeet caused vasodilation in control brain slices and vasoconstriction in brain slices from SAH rats. Paxilline, a blocker of large-conductance Ca^{2+}-activated K+ (BK) channels, greatly diminished both EFS-induced vasodilation and vasoconstriction in brain slices from control and SAH rats, respectively. Interestingly, we also observed an increase in the magnitude of spontaneous astrocytic Ca^{2+} oscillations in brain slices from SAH animals. The peak amplitude of spontaneous astrocytic Ca^{2+} oscillations in brain slices after SAH was ~490 nM compared to ~ 320 nM in brain slices from control animals. Our data are consistent with a model in which SAH increases the amplitude of spontaneous astrocytic Ca^{2+} oscillations leading to increased activity of endfoot BK channels and elevation of basal extracellular K+ in the restricted perivascular space between astrocytic endfeet and parenchymal arteriolar myocytes. This SAH-induced elevation in basal perivascular K+ combined with further K+ efflux stimulated by neuronal activity elevates K+ above the dilation/constriction threshold, switching the polarity of arteriolar responses from vasodilation to vasoconstriction. This inversion of NVC may contribute to decreased cerebral blood flow and the development of delayed ischemic neuronal deficits following SAH.

Supported by the Totman Medical Research Trust, Peter Martin Brain Aneurysm Endowment and the NIH (P01 HL095488, R01 HL078983, R01 HL078983-05S1, R01 HL044455 and P30 RR032135/P30 GM103498).

Poster #38

p38 MAP Kinase Signaling in Myeloid Cells Controls Autoimmune Disease of the CNS in a Sex-Specific Manner

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Multiple sclerosis (MS), a chronic inflammatory disease of the central nervous system (CNS), is the most common disabling neurological disorder affecting young adults. Sexual dimorphisms in severity and incidence of autoimmune diseases such as MS and lupus are well established, but poorly understood. Sex-specific disease modifying therapies (DMTs) are clearly warranted, but the mechanistic insight for their design is lacking. Using an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), we have previously shown that pharmacological inhibition of p38 MAP kinase (MAPK), a central regulator of inflammatory processes, prevents disease in C57BL/6 mice.

We now show that pharmacological inhibition of p38 MAPK only prevented EAE in female, but not male mice. To determine the cell types where p38 activity is required, we generated mice lacking p38alpha MAPK in T cells or myeloid cells (macrophages and microglia); since these cell types contribute to EAE and MS pathogenesis. T cell-specific deletion of p38alpha had no significant effect on EAE in either sex. In contrast, deletion of p38alpha in myeloid cells resulted in protection in females, but not males, suggesting that the sexual dimorphism in the EAE response to pharmacological blockade of p38 occurs within the myeloid cell compartment. Furthermore, male macrophages expressed higher levels of the gamma and delta isoforms of p38 MAPK, providing a male-specific mechanism that may bypass the requirement for p38alpha. These findings reveal important mechanisms underlying sex differences in autoimmune disease, and suggest that the p38 MAPK pathway may present targets for sex-specific DMTs.

Poster #39

Calcium-Sensitive Potassium Channels in the Decreased Myogenic Tone of Pial Arteries in a Genetic Model of Cerebral Ischemic Small Vessel Disease

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Small vessel disease (SVD) of the brain accounts for 25-30% of ischemic stroke, and refers to pathological processes that affect the structure and function of cerebral arteries. The Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is an archetypal monogenetic form of SVD. In a novel transgenic mouse model for CADASIL, a compromised cerebral blood flow and neurovascular coupling has been shown. This study investigates the consequences of CADASIL mutation on the function of mouse posterior cerebral arteries (PCAs). The relationship between intravascular pressure and diameter was determined in isolated PCAs and the function of potassium channels investigated. Elevation of intravascular pressure to 60 mmHg resulted in a reduced constriction of isolated PCAs from CADASIL mice. A reduced passive diameter, compared to control mice, was also observed. However, relaxation to NS309, an activator of endothelial small (SK) and intermediate (IK) conductance calcium-sensitive potassium (KCa) channels, was maintained suggesting a conserved endothelial KCa function. Moreover, blocking SK, IK and smooth muscle large conductance calcium-sensitive (BK) channels constricted CADASIL PCAs to the same extent in CADASIL mice compared to control mice. In contrast, endocytosis of voltage-dependent potassium (Kv) channels, via activation of epidermal growth factor (EGF) by addition of heparin-binding EGF (HB-EGF), resulted in an attenuated constriction of PCAs from CADASIL mice compared to control mice. In conclusion, the reduced tone development and reduced passive diameter in CADASIL mice support previous findings. In addition, KCa channel function is preserved in PCAs from CADASIL mice, whereas Kv channels regulation may play a critical role in the decreased myogenic tone observed.

Poster #40

Involvement of TRPM4 in Pressure- and Agonist-Induced Vasoconstriction in the Cerebral Microcirculation

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Cerebral parenchymal arterioles (PAs) play a critical role in regulating blood flow and perfusion pressure in the brain. These arterioles are heavily influenced by neurons and astrocytes through neurovascular coupling to maintain continuous supply of oxygen and glucose. They are substantially more sensitive to mechanical and chemical stimulants compared to cerebral arteries on the surface of the brain, i.e. the pial arteries. Intravascular pressure, an essential vasomotor stimulant, causes membrane depolarization and vasoconstriction (myogenic tone). Previous research in our laboratory indicated the involvement of a transient receptor potential channel (TRPM4) and purinergic receptors (P2Y4 and P2Y6) in myogenic tone development of pial arteries and PAs, respectively. The current study aims to investigate the relationship between TRPM4 and P2Y receptors, and their contribution to myogenic tone development in PAs. Here we report that TRPM4 mRNA signal is present in PAs. In functional studies, the selective TRPM4 channel blocker 9-phenanthrol substantially inhibited myogenic tone of endothelium-denuded PAs with an IC_{50} of 28 μM. Suppression of TRPM4 expression using siRNA constructs significantly reduced myogenic constriction; PAs treated with control siRNA and TRPM4 siRNA developed 45% ± 2% and 33% ± 4% myogenic tone, respectively. Moreover, 9-phenanthrol (30 μM) attenuated P2Y4 (UTPγS, 0.5 μM) and P2Y6 (MRS2693, 1 μM) agonist-induced vasoconstriction of denuded PAs by 67% and 68%, respectively. Consistent with this finding, TRPM4 channel activity in myocytes isolated from PAs was significantly elevated by UTPγS (0.5 μM). These results indicate that TRPM4 contributes to pressure-induced, purinergic receptor-mediated vasoconstriction of PAs.

Poster #41

Dietary Acetate Supplementation as a Means of Inducing Glioma Stem Cell Growth Arrest

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Glioma, the most common primary brain tumor of the adult central nervous system, is associated with a poor prognosis due, in part, to the presence of chemoradiotherapy-resistant glioma stem-like cells responsible for inevitable post-surgical recurrence. N-acetyl-L-aspartate (NAA), one of the most concentrated metabolic sources of acetate in the brain, and aspartoacylase (ASPA), the enzyme responsible for NAA degradation, are significantly reduced in glioma tumors. NAA-derived acetate is converted to acetyl coenzyme A via acetyl-CoA synthetase (AceCS) for use in lipogenesis, protein/histone acetylation, and the TCA cycle. We propose that glyceryltriacetate (GTA), a FDA approved food additive with "generally regarded as safe" status, may be an effective means of reducing glioma growth via restoration of acetate levels.

The effect of GTA on the growth of both established (Hs683, HOG) and stem-like (grade II OG33, grade III OG35) oligodendroglioma cell lines was assessed. In vitro, GTA induced growth arrest in all cells examined (i.e., increased proportion of cells in G_0/G_1 and reduced S phase cells by flow cytometry of propidium iodide labeled cells 24 hours after treatment and unbiased trypan blue exclusion based cytometry up to 5 days post-treatment). Growth arrest was not associated with apoptosis (lack of cleaved poly ADP-ribose polymerase immunolabeling), but differentiation (increased CNPase expression). ASPA expression was greater in stem-like cells when grown in stem cell media than differentiation media and was decreased in GTA-treated OG35 cells. Interestingly, GTA did not decrease ASPA expression in OG33 cells, but induced a novel 26 kDa ASPA isoform. ASPA and AceCS1 were co-localized within the nucleus of OG33/35 cells maintained in stem cell media. Nuclear, but not cytosolic, ASPA expression was decreased upon GTA addition in stem cell media, but not differentiation media. Finally, the effect of GTA on orthotopically grafted lentivirally transduced, luciferase expressing OG33 and OG35 cells was assessed. Bioluminescence and tumor volume was reduced in GTA treated mice. These data suggest that the nuclear ASPA/AceCS1 co-localization provides acetate for histone acetylation to maintain cells in a progenitor/stem-like state and that decreased ASPA promotes gliomagenesis. *Inasmuch as infants with Canavan Disease, a leukodystrophy due to ASPA mutation,* treated with high dose GTA showed no significant side effects, GTA may prove an effective therapy to prevent recurrence by inducing growth arrest/differentiation of glioma stem-like cells.

Poster #42

Activation of 5-ht4 Receptors in the Colonic Mucosa Elicits 5-HT Release, Goblet Cell Degranulation and Chloride Secretion, While Accelerating Propulsive Motility And Attenuating Visceral Hypersensitivity

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5-HT4 receptor (5HT4R) agonists promote colonic propulsive motility and attenuate visceral pain, yet concerns regarding adverse side effects have restricted their availability. Despite their therapeutic effectiveness, the distributions and functions of 5-HT4 receptors, particularly in the mucosa of the intestines, have not been well described. We tested the hypotheses that (1) 5HT4R agonists can activate responses in colonic epithelial cells, and (2) luminal application of 5HT4R agonists in the colon promotes propulsive motility and attenuates visceral hypersensitivity.

Mucosal expression of 5HT4R was evaluated by reverse transcriptase-PCR in mouse and human specimens, as well as sections from 5-HT4R(BAC)-eGFP mice. Continuous in vitro amperometry with diamond microelectrodes, periodic acid-Schiff (PAS)-Alcian Blue (AB) histology, and Ussing chamber short-circuit current measurements were used to study 5-HT, mucus, and Cl- secretion, respectively. Propulsive motility was measured in an ex vivo guinea pig distal colon model of fecal pellet propulsion, and visceromotor responses to colorectal distension recorded in a conscious rat model of acetic acid-induced colonic hypersensitivity. 5HT4R compounds included cisapride, tegaserod, naronapride, SB204070, and GR113808.

Mucosal 5-HT4R expression was present in the small and large intestines. In the distal colon, 5-HT4Rs appeared to be expressed by all epithelial cells, including 5-HT-containing enterochromaffin (EC) cells, mucin-immunoreactive goblet cells and enterocytes. Application of a 5HT4R agonist to the mucosal surface evoked 5-HT release from EC cells, goblet cell degranulation, and Cl- secretion, and these responses were blocked by 5HT4R antagonist administration. Intraluminal administration of 5HT4R agonists accelerated propulsive motility, and this effect was inhibited by a selective 5HT4R antagonist. Both oral and intracolonic delivery of 5HT4R agonists attenuated visceral hypersensitivity, yet intracolonic administration was more potent than oral administation, and the 5HT4R antagonist blocked this effect.

In conclusion, activation of colonic mucosal 5HT4Rs causes epithelial responses that could alleviate constipation, and mediates prokinetic and anti-nociceptive actions. These findings suggest mucosal 5-HT4 receptors as a target to promote colonic propulsive motility and attenuate visceral pain, while restricting systemic bioavailability and resulting adverse side effects.

Poster #43

Mechanism of Pituitary Adenylate Cyclase Activating Polypeptide (PACAP)-Induced Dilation of Middle Meningeal Artery: Role of ATP-Sensitive Potassium (K_{ATP}) Channels

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Migraine is a complex neurological condition that presents as intense episodic unilateral headaches accompanied by nausea, photophobia, phonophobia and other neurological symptoms. The causes of migraine headache appear multifactorial but may involve dilation of the meningeal vasculature. Intravenous injection of the neuropeptide PACAP induces migraine-like symptoms and dilation of the middle meningeal artery (MMA) in both healthy and migraine patients. We have recently shown that PACAP dilates isolated pressurized MMA from the rat with a 1000-fold higher potency compared to other vascular beds via activation of the high affinity Gs/cAMP-coupled PAC1 receptor. It has also been shown that the cAMP/PKA pathway can activate K_{ATP} channels in vascular smooth muscle leading to vasodilation. In the present study our goal was to decipher if picomolar concentrations of PACAP activate K_{ATP} channels to induce MMA dilation. The K_{ATP} channel opener cromakalim induced a concentration-dependent dilation of MMA with an EC50 of 100 nM, providing evidence of functional K_{ATP} channels. Further, PACAP-induced MMA dilation was abolished by the K_{ATP} channel blocker glibenclamide. These observations indicate that PACAP dilates MMA via activation of vascular KATP channels which may play role in the etiology of migraine.

Poster #44

Chronic Stress Elevates Capacity And Decreases Spontaneous Contractions In Mouse Urinary Bladder

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Hinman Syndrome is one of the most severe forms of acquired voiding dysfunction, usually diagnosed in children. This syndrome has a significant psychological component, and is characterized by neuropathic-like bladder dysfunction. However, the pathophysiology of Hinman Syndrome is poorly understood. Due to the psychological basis of Hinman Syndrome, we hypothesized that the social stress mouse model may help determine how psychological factors impact bladder physiology in conditions like Hinman Syndrome. 6-week old C57BL6 male mice were randomized into three groups: control, mild stress and severe stress. Mice were stressed using the social defeat/resident intruder/bullying model, wherein they were exposed to an aggressor mouse for 1 h/day (mild) or 24 h/day (severe). After 2 weeks, animals were sacrificed, bladders were harvested and ex vivo pressure-volume curves were generated during constant infusion of PSS (1.8 ml/hour). Mild and severe social stress significantly increased ex vivo bladder capacity. While all bladders exhibited spontaneous phasic contractions (SPC's) as pressure increased, the amplitude, area and frequency of SPC's was significantly reduced in bladders from mild stressed mice as compared to control. Inhibition of BK and SK channels by apamin (300 nM) and paxilline (1 μM), respectively, lead to the recovery of spontaneous contractile activity in mild stress bladders. These data suggest that mild stress elevates BK and SK channel activity and/or expression, thus decreasing spontaneous bladder contractions in mice. Also, increased BK/SK activity or expression may underlie a more relaxed urinary bladder that allows for greater capacity.

Poster #45

Cerebral Vascular Dysfunction Following Traumatic Brain Injury

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Traumatic brain injury (TBI) affects more than 1.7 million people in the United States annually and has a major impact on public health. Cerebral autoregulation is altered in humans after TBI and in animal models, reflecting a fundamental impairment in myogenic tone (the active contractile response elicited by an increase in pressure). We hypothesize that TBI leads to abnormal increases in cerebral endothelial Ca2+ signaling leading to decreased cerebral vascular tone. A fluid percussion injury model of brain injury was used to producemoderate TBI in adult Sprague-Dawley rats and animals were studied 24 h after injury.Magnetic resonance imaging (MRI) demonstrates brain damage and edema involving both the ipsilateral and contralateral sides of the cerebral cortex. Cerebral arteries from TBI animals exhibited decreased cytosolic smooth muscle (SM) Ca2+ and myogenic tone in response to step-wise increases in intravascular pressure compared to controls. Inhibition of nitric oxide (NO) synthase with Nω-L-arginine (L-NNA) induced larger constriction in TBI compared to controls.L-NNA also restoredboth myogenic toneand SM Ca2+observed after TBI. Dilations to sodium nitroprusside, a NO donor, were enhanced after TBI indicating that vascular SM cells are hypersensitive to exogenous NO. Bioavailability of NO assessed via 4,5-diaminofluorescein (DAF-2 DA) is increased in TBI animals compared to controls. These resultssuggest that abnormalities in nitric oxide vasodilatory pathways are involved in mechanisms of cerebrovascular dysfunction following TBI; this information may ultimately lead to novel therapeutic targets that improveclinical outcomes.

Poster #46

TRPV4 Sparklets - Elementary Ca^{2+} Signals Underlying Endothelial-Dependent Vascular Function

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Endothelial cells (ECs) regulate vascular tone and maintain barrier function. Elevated intracellular Ca^{2+} is central to EC function. We report the discovery of local Ca^{2+} signals ("sparklets") in the intact vascular endothelium of resistance arteries that represent Ca^{2+} influx through single TRPV4 (Transient receptor potential vanilloid 4) channels. We used spinning disk confocal imaging for recording the Ca^{2+} sparklets in third-order mouse mesenteric arteries (MAs). Activation of TRPV4 sparklets with GSK1016790A (GSK, TRPV4 agonist, 10 nM) was cooperative, with fewer than two sparklet sites (<8 channels) per cell. Diameter studies in pressurized mouse MAs showed that GSK caused maximal, nitric oxide (NO)-independent dilation through activation of EC intermediate (IK)- and small (SK)-conductance, Ca^{2+}-sensitive K+ channels (n=5-7). GSK also increased IK- and SK-channel current densities in isolated ECs (n=5). These results support a novel concept that Ca^{2+} influx through a single TRPV4 channel is leveraged by the combined amplifier-effect of cooperative channel activation and high Ca^{2+} sensitivity of IK/SK channels to cause vasodilation, and thus provide a new paradigm for transcellular signaling.

Sponsors

Financial support for this meeting was provided by a Chapter Grant from the Society for Neuroscience and from 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.