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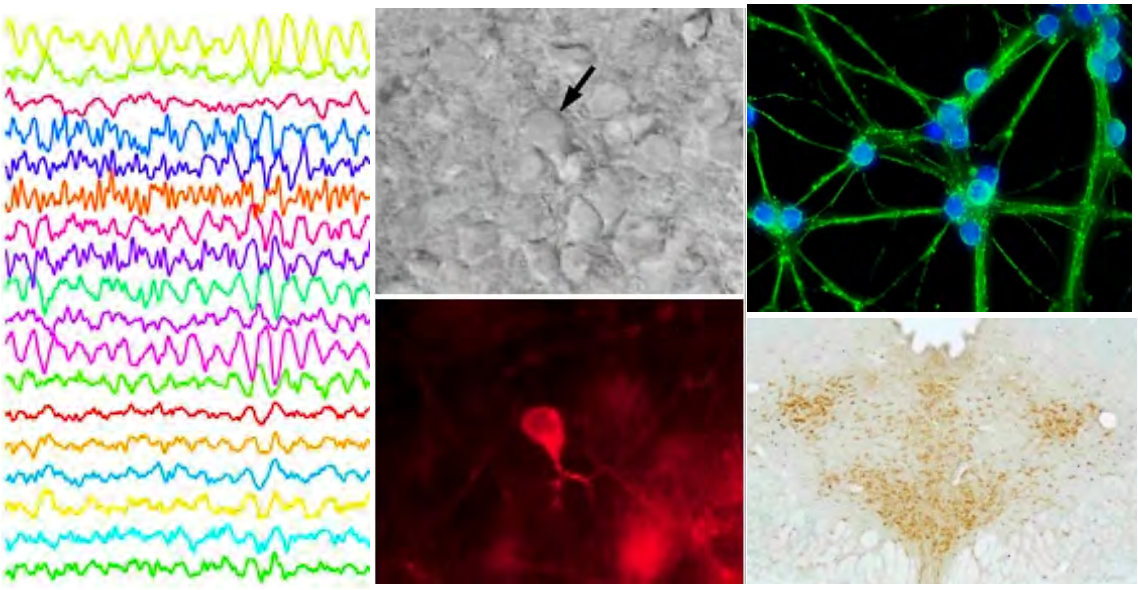

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SOCIETY FOR NEUROSCIENCE

DECEMBER 7, 2011
FORT COLLINS MARRIOTT

FRONT RANGE NEUROSCIENCE GROUP

Annual Meeting: December 7, 2011

The Place: **Fort Collins Marriott** (350 E. Horsetooth Rd.)

Registration (payable at the door) - 10:00am-6:30pm

*Contact stuart.tobet@colostate.edu for registration information

* * PROGRAM * *

10:30-11:00am – Cool Tools Data Blitz

11:00-noon – Research Data Blitz

Noon-3pm – Lunch, Posters, Vendors!

3-4pm – Trainee presentations

Jodi Lukkes (UC Boulder)
Reagan Pennock (CSU)
Erika Ross (UD)
Elliott Forney (CSU)

4:00-4:30pm – Coffee break, stretch

4:30-5:30pm – Keynote:

Dr. Bertil Hille, PhD, University of Washington

Title: G-protein Coupled Receptors Rule the Mind Through Phosphoinositide Signals

5:30-6:30pm – Awards, door prizes, reception!

SfN http://FRNG.colostate.edu SfN

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Find out more: Stop by our BAW Table during the POSTER Session of the FRNG conference

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Acknowledgements:

Cover Page: Designed by Christina Dennison
Scientific images provided *by Jodi Lukkes (UC Boulder), Reagan Pennock (CSU), Erika Ross (UD) and Elliott Forney (CSU).* Details will be in their oral presentations. The FRNG website (http://FRNG.colostate.edu) was created by Leif Saul in 2005 – see more images on our website. Thanks again to Leif for creating our electronic abstract submission system!!

Special Thanks!

Special thanks to all of you that submitted abstracts for oral and poster presentations! We particularly thank the judges for the poster contest!! – and to Shane Hentges for managed the herculean task of organizing the judging operation for the meeting – no easy task!!!

Special thanks to the vendors listed in this program. These companies have declared by their contributions both in dollars and prizes that they value Front Range Neuroscience Group business. We encourage you to buy from these vendors that support you.

Special thanks to our Platinum Level Industry Supporters: Martek Biosciences Corporation (now a division of DSM), Leica Microsystems, Olympus America, and Fisher Scientific. In addition, special thanks to the Hilton Fort Collins for stepping up to a platinum level of support in providing the ideal venue and extra contributions, and to Kate Reid in particular for help in making this all possible.

Special thanks to the University departments and programs that provided financial support to help make the meeting possible; in particular Colorado State University, the University of Wyoming, the University of Colorado at Boulder and the Eleanor Roosevelt Institute of the University of Denver.

Special thanks to the graduate student organizing committee for creating and polishing the program and fixing the details, and in particular for creating the program book. This includes Krystle Frahm and Christina Dennison from CSU, Dori R. Pitynski and Colleen M. Cassidy from Univ Wyoming, Jenn Whitesell, and Emily Aurand from UCD Health Science Center, Nina Donner and Jessica Babb from UC-Boulder and Josie Gray from DU. And additionally to Erin Bisenius, Brett Beal and the first year MCIN students Mallory, Michael, An and Vanessa for helping with attendee registration.

Special thanks to Dr. Tom Finger and the Rocky Mountain Regional Neuroscience Group for partnering to make this a great Front Range event.

Special thanks to you, the attendees, for making this a meeting that we can be proud to hold on a regular basis, and for forming Front Range Neuroscientists into a vibrant and interactive Community!

Stay tuned for information on our FRNG Website that helps us communicate position openings, course offerings, seminars and a whole lot more!!!

Sincerely yours,

The Front Range Neuroscience Steering Committee,
Shane Hentges, Susan Tsunoda, Qian-Quan Sun, Serge Campeau, Nancy Lorenzon, Mark Basham, Sondra Bland, Patrick Burns and Stuart Tobet.

Keynote Speaker

Dr. Bertil Hille, PhD
University of Washington

Title: G-protein Coupled Receptors Rule the Mind Through Phosphoinositide Signals

The lab is interested in cell signaling by ion channels, neurotransmitters and hormones acting through G-protein coupled receptors and intracellular calcium. The talk will be much about the molecular and biophysical basis of GPCR signaling, but placed in the neurobiological context that GPCR signaling changes mental state and perception of reality.

'DATA' BLITZES: Front Range Style

COOL TOOLS

1) David Gire (UCD) "Optogenetics in transgenic animals and engineering solutions to record activity from optically stimulated neurons"

2) Mike Baratta (UCB) "The use of optogenetics"

3) Tom Chen (CSU) "Deeper understanding or biological signaling using silicon chips"

4) Randy Bartels (CSU) "High speed three dimensional nonlinear optical imaging"

DATA BLITZ

1) Scott Barbee (University of Denver) "Functions for neuronal RNPs ("neuronal granules") and miRNAs in the regulation of local mRNA translation and synaptic plasticity."

2) Justin E Hellwinkel (UCB) "Exercise can facilitate contextual, but not auditory fear, as assessed by an animals freezing behavior after fear conditioning"

3) Jon Murphy (UCD-AMC) "Live cell calcium imaging in single dendritic spines and its regulation by AKAP79/150"

4) Susan Tsunoda (CSU) "A Novel Role for K+ Channels in Synaptic Homeostasis"

5) Beth Chagnon and Dori Pitynski-Dor (UW) "The Pallid Bat: Complex Processing in an Auditory World"

6) Chris Lowry (UCB) "An automated blood sampling system for assessment of diurnal, ultradian, and stimulus-induced activation of the HPA axis"

7) Ernie Salcedo (UCD) "CAGE MATCH! Effect of Rodent Housing Conditions on Olfactory Anatomy and Aggressive Behavior"

8) Jozsef Vigh (CSU) "Plasticity of the synapses in the retina"

9) Julia Dee Campbell, (UCB) "Honey, turn the TV up! Hearing loss and the adult brain"

10) Dan Raible (UCD) "JaK/STAT Inhibition to Prevent Post-Traumatic Epileptogenesis"

11) Zhen Lu (UW) "Genetic screen identifies two thiol reductase enzymes that decrease mutant huntingtin levels in cultured cells"

ORAL PRESENTATIONS

Jodi L. Lukkes, Christopher A. Lowry
THE EFFECTS OF POST-WEANING SOCIAL ISOLATION ON SEROTONERGIC SYSTEMS AND BEHAVIOR, University of Colorado, Boulder, CO, USA. Exposure to stressful experiences, such as social isolation, during adolescence can contribute to vulnerability to stress-related psychiatric disorders during adulthood. My previous studies have shown that post-weaning social isolation in male rats causes an up-regulation of corticotropin-releasing factor (CRF) type 2 receptor levels in the dorsal raphe nucleus (DR), alters CRF-mediated serotonin release in the nucleus accumbens (NAc), and increases social anxiety-like and fear behavior in adulthood, which can be attenuated with antagonism of CRF type 2 receptors in the DR. These findings suggest that the social isolation-induced alterations in fear, anxiety, and stress-related behavioral responses could be due to sensitization of stress-related, CRF-dependent activation of a DR-NAc serotonergic circuit. Therefore, we examined how post-weaning social isolation, in combination with a subsequent stressor such as social defeat in males or a challenge with the anxiogenic drug, N- methyl-beta-carboline-3-carboxamide (FG-7142) in females, affects c-Fos expression in topographically organized subpopulations of serotonergic neurons in the DR in adulthood using dual immunohistochemical staining for c-Fos and tryptophan hydroxylase. Post-weaning social isolation sensitized female rats to anxiogenic drug-induced increases in c-Fos expression in serotonergic neurons in the DR. Furthermore, post-weaning social isolation increased anxiety and promoted a reactive emotional coping style in male rats. These data suggest that post- weaning social isolation alters the effects of stress-related stimuli on serotonergic systems, which have been implicated in the pathophysiology of stress-related neuropsychiatric disorders.
Acknowledgements: The project described was supported by Award Numbers F32MH084463 (JLL) and R01MH086539 (CAL) from the NIMH. C.A. Lowry is supported by a 2010 NARSAD Young Investigator Award and is currently supported by an NSF CAREER Award (NSF-IOS #0845550).

Reagan L. Pennock, Matthew S. Dicken, and Shane T. Hentges
PRESYNAPTIC GI/O COUPLED RECEPTORS RESIST ACUTE DESENSITIZATION
Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.
$G_{i/o}$ coupled receptors located in the somato-dendritic region of neurons modulate neuronal activity through a direct inhibition of firing, while the same receptor located on presynaptic terminals modulates activity by inhibiting neurotransmitter release. Previous studies suggest that the location of a $G_{i/o}$ coupled receptor on a neuron may determine that receptors resistance to acute desensitization, however these studies generally focus on a single type of $G_{i/o}$ coupled receptor and are spread across many regions of the brain. To further investigate how compartmentalization correlates with $G_{i/o}$ coupled receptor desensitization, whole cell voltage clamp experiments were performed in proopiomelanocortin (POMC) neurons of the hypothalamus, which are regulated both pre- and postsynaptically by multiple $G_{i/o}$ coupled receptors. Agonists for the mu opioid receptor (MOR), $GABA_B$ receptor ($GABA_BR$) and nociceptin receptor (ORL-1) inhibited POMC neurons directly through the induction of a postsynaptic potassium conductance as well as inhibited the release of GABA onto POMC neurons presynaptically, which was measured as a reduction in the amplitude of evoked inhibitory postsynaptic currents (eIPSCs). The potassium current induced by all three agonists desensitized rapidly in the prolonged presence of agonist, reaching a steady state within minutes of the peak effect. No desensitization of the inhibition of neurotransmitter release by MOR or ORL-1 was detected under a similar exposure to agonist, however $GABA_BR$ mediated inhibition did desensitize in ~25% of recordings. Thus, resistance to desensitization is a property shared by $G_{i/o}$ coupled receptors found on presynaptic terminals. Further, the fraction of presynaptic $GABA_BRs$ that do desensitize suggest that resistance to desensitization may be conferred by a property of the receptors and not an intrinsic property of the terminals on which they are located.
Supported by NIH Grant R01DK0798749 (STH)

Erika K. Ross, Heather Wilkins, Whitney Hulick, Danielle Kirchoff, Aimee Winter, Nathan Duval, David Patterson, and Daniel Linseman

A NON-DENATURED WHEY PROTEIN SUPPLEMENT (IMMUNOCAL®) PROVIDES NEUROPROTECTION FROM MITOCHONDRIAL OXIDATIVE STRESS *IN VITRO,* DELAYS DISEASE ONSET AND PREVENTS GSH DEPLETION IN THE HSOD1^{G93A} MOUSE MODEL OF ALS Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver; Research Service, Veterans Affairs Medical Center, Denver, Colorado

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that affects the α-motor neurons of the spinal cord. Many studies indicate that mitochondrial oxidative stress (MOS) is a principal mechanism underlying the pathophysiology of this disease. Here, we investigated a unique whey protein supplement (Immunocal®) to determine its neuroprotective efficacy in several *in vitro* models of MOS and in an *in vivo* mouse model of ALS. This non-denatured whey supplement contains cystine which is an oxidized form of cysteine, an important precursor for the synthesis of the essential endogenous antioxidant, glutathione (GSH). In primary cultured cerebellar granule neurons (CGNs), pre-incubation with Immunocal® completely protects against HA14-1, an inhibitor of the pro-survival Bcl-2 protein. This effect is prevented by co-incubation with the gamma-glutamylcysteine ligase inhibitor, buthionine sulfoximine, demonstrating that the *de novo* synthesis of GSH underlies the neuroprotective mechanism of Immunocal®. Additionally, Immunocal® displays significant protection against an array of MOS-inducing agents, including sodium nitroprusside, copper, and aluminum, supporting its ability to upregulate mitochondrial antioxidant capacity. In accordance with these findings in CGNs, Immunocal® also significantly protects CHO cells from MOS evoked by overexpression of amyloid precursor protein. Most interesting are our findings in the G93A mutant SOD1 mouse model of ALS. These mice were given Immunocal® (10% solution in drinking water) *ad libitum*, beginning at 60 days old. Although no effect on overall survival was observed, Immunocal®-treated mice display a significant (~10 day) delay in disease onset, compared to mutant control mice. Moreover, Immunocal®-treated mice showed a highly significant decrease in the rate of decline in grip strength. Finally, using HPLC-ECD we found that whole blood GSH was depleted by nearly 50% in end-stage G93A mutant SOD1 mice, and that this reduction was essentially prevented in mutant mice receiving Immunocal®. These findings suggest that sustaining GSH by supplementation with Immunocal® may help to mitigate the progression of ALS through suppression of MOS. (Supported by a Merit Review grant from the Department of Veterans Affairs and NIH R01NS062766 to D.A.L.).

Elliott Forney and Chuck Anderson

MODELING AND CLASSIFICATION OF EEG BY FORECASTING WITH RECURRENT ARTIFICIAL NEURAL NETWORKS, Dept Computer Science, Colorado State University, Fort Collins, CO

A Brain-Computer Interface (BCI) is a system that allows a user to operate a computerized device by voluntarily manipulating their mental state. BCI bypass our innate motor-based means of communication by directly observing changes in neural activity. This may yield an exciting new form of communication, particularly for those who suffer from disabilities that make interaction with the outside word difficult, such as amyotrophic lateral sclerosis, high-level spinal cord injury and some forms of stroke.

Recently, there has been significant interest in building non-invasive BCI systems using electroencephalography (EEG). In these approaches, an array of electrodes is placed on the surface of a subject's scalp in order to monitor brain activity. Machine learning and pattern analysis algorithms can then be used identify changes in mental state and issue the appropriate commands to the device that the user wishes to control. Although a number of research groups have demonstrated that this approach can deliver working BCI systems, current approaches do not perform well enough for use in many practical, real-world applications.

We assert that current approaches are often limited by the use of purely frequency-based feature representations and by linear classification classification algorithms. In order to address these limitations, we have developed an algorithm for identifying patterns in EEG that utilizes

versatile learning machines known as Recurrent Artificial Recurrent Neural Networks (RNN). RNN consist of a number of simple computational units with weighted interconnections. An RNN can be trained to map inputs to outputs by adjusting the strength of the connections between the computational units. RNN also contain delayed feedback connections which gives them an intrinsic memory and the ability to learn complex spatiotemporal patterns.

In order to identify a user's mental state through EEG, we first train a separate RNN to model sample EEG recorded during each mental state by forecasting the signal a single step ahead in time. Thus, if we have K imagined mental states we train K different RNN. Each of these RNN models can then be thought of as an expert at predicting EEG produced during each mental state. Previously unseen EEG can then be identified by applying each RNN and assigning the label associated with the model that was able to best predict the signal.

We test this approach on EEG recorded from five subjects, two of which are able-bodied, two with high-level spinal cord injuries and one with severe multiple sclerosis. A cue was presented to each subject on an LCD screen instructing them to perform one of four imagined mental tasks: imagined right hand movement, counting backward from 100 by 3's, silently sing a favorite song and visualization of a tumbling cube. Although our preliminary analysis is performed offline, a BCI can be operated in this fashion by associating a command with each imagined mental task. For example, imagined right hand movement may turn a wheelchair to the right while silently singing a song may turn it to the left. Although cumbersome at first, this technique may become second-nature with extended periods of practice.

The application of our classification algorithm to these datasets indicates that a BCI user may be able to communicate as many 34.5 bits per minute when selecting between four tasks with decisions made every second. On average, these subjects are able to communicate 13.2 bits per minute, suggesting that this technique outperforms a number of other state-of-the-art approaches. Additionally, we observe that placing a feedback loop between the inputs and outputs of a trained RNN, forming an autonomous and self-driven system, produces rich and long-term dynamics that strongly resemble true EEG.

POSTER PRESENTATIONS

Cognition and Behavior

1) The disruptive effect of instrumental non-contingency on executive functioning. T Gupta, R Toll.

2) Increased anxiety-like behavior in fibroblast growth factor 8-deficient mice. LR Brooks, CL Enix, SC Rich, CA Lowry, PS Tsai.

3) BXD recombinant inbred mice with low brain iron exhibit a reduced dopaminergic-mediated synaptic transmission in the CA1 region of the hippocampus. AB Breton, JA Fox, MP Brownson, M.D. McEchron.

Development

4) Real-Time Nitric Oxide Release Measurements: Towards Understanding Cell Migration in Brain Tissue. CM Bishop, MM Reynolds.

5) Adult amphetamine-induced dopamine release in the medial prefrontal cortex and nucleus accumbens following adolescent social defeat in rats. AR Burke, GL Forster, AM Novick, CL Roberts, MJ Watt.

6) Supplementation of the maternal diet with free sialic acid increases neurite growth and white matter maturation in the offspring. AE Garrison, MJ Weiser, JP Zimmer, CM Butt.

7) Tbx5 function is dispensable for cell proliferation in zebrafish development. LE Parrie, EM Renfrew, MA DeMiranda, DM Garrity.

8) The conserved P-body component HPat/Pat1 regulates synaptic structure at the larval Drosophila neuromuscular junction. SJ Pradhan, K Nesler, LM Rozeboom, Y Kato, A Nakamura, M Ramaswami, SA Barbee.

9) The miRNA pathway controls rapid activity-dependant changes in synapse structure at the Drosophila neuromuscular junction. KR Nesler, RI Sand, BA Symmes, ML Ritz, SA Barbee.

10) Gonadal steroid influences on the development of perinatal sex differences in the preoptic area. BT Searcy, P Kumar, MS Stratton, SA Tobet.

11) Mechanical Properties of Hyaluronic Acid-Poly(Ethylene Glycol)-based Hydrogels for the Development a Synthetic Brain Extracellular Matrix. ER Aurand, KB Bjugstad.

12) Vasculature within the paraventricular nucleus of the hypothalamus: Development, location and GABA signaling. KA Frahm, MJ Schow, Q Zhang, CM Eitel, SA Tobet.

13) Embryonic GABA-B receptor blockade alters adult hypothalamic structure, and anxiety- and depression-like behaviors in mice. M Stratton, T Budefeld, G Majdic, SA Tobet.

14) Connexin 35 in zebrafish spinal cord development**.** TC Martin, AB Ribera.

Disorders of the Nervous System

15) Bcl-2 is a novel interacting partner for the 2-oxoglutarate carrier and a key regulator of mitochondrial glutathione. HM Wilkins, K Marquardt, LH Lash, DA Linseman

16) Acute regulation of MCT1 function in cerebrovascular endothelial cells by cAMP dependent vesicular trafficking. JP Smith, AL Uhernik, BN Nuanez, KT Darcy, M Sneve, Z Liu, LR Drewes.

17) Nutraceuticals offer protection from neuronal apoptosis resulting from nitrosative and oxidative stress in rat cerebellar granule neurons. JJ Gray, TC Sutcliffe, AN Winter, NA Kelsey, DA Linseman.

18) Neuroprotective effects of supra-nutritional selenium in a mouse model of Huntington's disease. J Chen, E Marks, J Molline, L Barrows, M Stiles, I Volitakis, A Bush, S Hersch, J Fox.

19) A novel method to incorporate nerve growth factor onto a polypyrrole coated nanowire scaffold for potential nervous tissue engineering applications. SL Bechara, KC Popat.

20) Subcortical auditory dys-synchrony affects cortical maturation in children with Auditory Neuropathy Spectrum Disorder. GJ Cardon, A Sharma.

21) Genetic and pharmacologic blockade of NF-kB prevents ongoing neuroinflammation in the MPTP model of Parkinson's disease. BR Trout, JA Miller, KA Kirkley, SA Safe, RB Tjalkens.

22) Effects of adolescent social isolation and acute anxiogenic drug treatment during adulthood on tph2 mRNA expression in female rats. JL Lukkes, JM Kopelman, NC Donner, MW Hale, CA Lowry.

23) Integrative physiology of antidepressant drug action**.** KF Dady, MW Hale, JL Lukkes, KJ Kelly, CL Raison CL, CA Lowry.

Neural Excitability, Synapse and Glia

24) Regulation of GABA and Glutamate Release from Proopiomelanocortin Neuron Terminals in Intact Hypothalamic Networks. MS Dicken, RE Tooker, ST Hentges.

25) ATP-dependent calcium signaling in striatal astrocytes is acutely sensitive to inhibition by structurally diverse cationic neurotoxicants. K Streifel, A Gonzales, B Trout, L Maxwell, B Mohl, S Earley, R Tjalkens.

26) Proopiomelanocortin neurons in the arcuate nucleus have inhibitory and excitatory subpopulations. BC Jarvie, ST Hentges.
27) Hydrogen peroxide mediates oxidative stimulation of L-type calcium channels in arterial smooth muscle. NL Chaplin, GC Amberg.

28) Site-directed metabolic biotinylation of AMPA receptors may perturb protein-protein interactions with TARPS. A Dudek, LM Stone-Roy, KM Partin.

29) Activity-dependent retrograde signaling lowers voltage-gated calcium current threshold in retinal bipolar cells**.** R Tooker, J Bramley, E Rozsa and J Vigh.

30) Kv2.1 non-conducting state is dependent on cell-surface density, independent of cell-surface localization, and present endogenously in cultured hippocampal neurons. PD Fox, RL Loftus, E Deutsch, MM Tamkun.

31) Design and Profiling of a series of AMPA receptor modulators. JE Harms, KM Partin, C Jamieson.

32) Direct CAMP binding and PKA phosphorylation share a common gating mechanism in HCN4 channels. Z Liao, J St. Clair, ED Larson, C Proenza.

33) β adrenergic regulation of If and heart rate in sinoatrial node myocytes is dependent upon AKAP tethered PKA. E Larson,Z Liao, J St. Clair, ML Dell'Acqua, C Proenza.

34) Kv2.1 cell surface clusters are insertion and retrieval platforms for ion channel trafficking at the plasma membrane. E Deutsch, AV Weigel, EJ Akin, P Fox, G Hansen, R Loftus, D Krapf, MM Tamkun.

35) A novel fluorescent protein and biotin tagged Nav1.6 channel allows analysis of voltage-gated sodium channel dynamics in hippocampal neurons. EJ Akin, AV Weigel, D Krapf, MM Tamkun.

36) An AKAP79/150:L-Type calcium channel interaction is required for CaN-NFAT nuclear signaling. JG Murphy, ML Dell'Acqua.

37) Phosphodiesterases form a cAMP “shield” to regulate the funny current, If, in mouse sinoatrial myocytes**.** JR St. Claire, Z Liao, E Larson, C Proenza.

Neuroendocrine

38) Elevated tph2 mRNA expression in an amygdala priming model of chronic anxiety. NC Donner, PL Johnson, SD Fitz, KE Kellen, A Shekhar, CA Lowry.

39) Deciphering the role of histone deacetylases in GnRH neuronal development. S Salian-Mehta, M Xu, Horn T, Mckinsey T, ME Wierman.

40) Gonadotrophin-releasing hormone system during pubertal transition of fibroblast growth facotr 8-deficient mouse. W Zhang, J Rochester, S Kavanaugh, P-S Tsai.

41) Localization of the expression of gonadotropin-releasing hormone like-molecule in a gastropod mollusk aplysia californica. L Jung, S Kavanaugh, P-S Tsai.

42) Opposite-sex cohabitation restores the aging GnRH system and promotes the morphological maturation of GnRH neurons in transgenic animals. JR Rochester, P-S Tsai.

43) Short term exposure of a high dose GnRH agonist and its effects on gonadotropin secretion in the anterior pituitary of adult male rats. BS Edwards, CS Asa, DC Skinner.

44) Gonadotropin-releasing hormone receptor in Aplysia californica. SI Kavanaugh, P-S Tsai.

45) Sex differences in the central control of HPA axis responses is dependent on stressor modality. JA Babb, CV Masini, HEW Day, S Campeau.

46) Anti-apoptotic role of ceramide synthase 6 (CerS6) in gonadotrope pituitary tumors. K Kiseljak-Vassiliades, M Xu, AJ Knox, KA Michaelis, BK Kleinschmidt-DeMasters, KO Lillehei, ME Wierman.

47) The nonpathogenic, saprophytic bacterium, Mycobacterium vaccae, selectively activates a subset of serotonergic neurons in the dorsal raphe nucleus in association with hypothermia in mice. PHW Siebler, MW Hale, JL Lukkes, CA Lowry.

Sensory and Motor Systems

48) Cross-modal re-organization in adults with mild hearing loss. J Campbell, L Durkee, A Sharma.

49) Mechanisms of cholinergic modulation of midbrain motor output: An optogenetic approach. EA Stubblefield, Gidon Felsen.

50) Sub-sensory mechanical noise input to ankle tendons improves movement detection. BL Tracy, AM Grossman, AA Amin, JL Uphoff, SK Anast, RJ Paxton.

51) Characterization of axo-axonic synapses in the piriform cortex of musculus. X Wang, Qian-quan Sun.

52) Risk, variability, and decision-making in goal-directed movements. MK O'Brien, AA Ahmed.

53) Movement adaptation under conditions of risk and instability. MC Trent, AA Ahmed.

54) Does actual metabolic cost decrease with motor learning? HJ Huang, R Kram, AA Ahmed.

55) Timing-dependent suppression of mitral cell output by inter-glomerular lateral inhibition targeted on external tufted cells. JD Whitesell, NE Schoppa.

56) GABA-induced calcium transients in juxtaglomerular neurons of the mouse olfactory bulb. PV Parsa, RD D'Souza, S Vijayaraghavan.

57) Peripheral anatomy of a distinct class of rapidly adapting sinus hair follicle afferents. CC Cassidy, CJ Woodbury.

ABSTRACTS

Cognition and Behavior

1) **The disruptive effect of instrumental non-contingency on executive functioning.**

T Gupta, R Toll. From the Department of Psychology and Neuroscience, University of Colorado at Boulder, Boulder, CO

Previous research has suggested that an inability to learn how to control one's environment (instrumental non-contingency) has negative effects on cognitive abilities, as demonstrated by impaired performance on subsequent cognitively demanding tasks. However, the nature of such cognitive impairment is poorly specified. The current study tested to the hypothesis that instrumental non-contingency leads specifically to disruption in cognitive control functioning. The sample consisted of undergraduate students (n=108) recruited from general psychology courses and randomly assigned to one of three groups: control, instrumental contingency (IC), or instrumental non-contingency (IN). Participants completed the color-word Stroop, a classic test of cognitive control, at baseline. Next, participants completed an instrumental learning task in which they either were able to learn how to control a noise stress (IC), were unable to learn how to control a noise stress (IN), or were not exposed to stress or instrumental learning demands (control). Finally, participants completed the color-word Stroop a second time. Results indicated that while the IC group showed practice-related improvements in Stroop performance, the IN group failed to show such improvements. These findings suggest that being unable to learn how to control one's environment causes disruption to executive functioning. These results provide support for behavioral therapies that encourage instrumental learning as a strategy for protecting the individual from the negative effects of stress, and suggest that such strategies specifically protect cognitive control abilities.

2) **Increased anxiety-like behavior in fibroblast growth factor 8-deficient mice.**

LR Brooks, CL Enix, SC Rich, CA Lowry, PS Tsai. From the Department of Integrative Physiology and Center for Neuroscience, University of Colorado, Boulder, CO.

Serotonergic systems modulate mood-, stress- and anxiety-related behaviors. Serotonergic (5-HT) neurons in the dorsal raphe nucleus (DRN) comprise multiple subpopulations with distinct anatomical locations, afferent inputs and efferent targets. Prior research has shown that 5-HT neurons are highly dependent on fibroblast growth factor (FGF) signaling for their prenatal development. Developmental deficiency in FGF signaling results in a significant loss of 5-HT neurons around the time of birth. However, it is unclear if specific populations of 5-HT neurons are impacted under FGF signaling deficiency, and whether these changes result in altered anxiety-like behavior in adulthood. To answer these questions, wild-type (WT) and Fgf8 heterozygous hypomorphic (Fgf8+/-) mice, which have roughly a 25% reduction of Fgf8 mRNA, were tested in the elevated plus-maze (EPM) for anxiety-like behavior. Further, we tested if their behavior is accompanied by an alteration in the organization of 5-HT neurons in the DRN. Adult male WT and Fgf8+/- mice were placed on the EPM for 5 minutes and scored for the following parameters: time spent in the open, closed and center areas, and total number and percent entries into each arm. Fgf8+/- mice spent significantly less time in and initiated fewer entries into the open unprotected arm than WT controls. There were no differences between genotypes in overall explorative activities as determined by the sum of all entries into either arm. This increased avoidance of the aversive properties of height and openness suggests that Fgf8+/- hypomorphic male mice have

increased anxiety-like behavior. Immunohistochemical staining of the serotonergic marker, tryptophan hydroxylase 2, revealed subtle changes in the organization of serotonergic neurons throughout the DRN, suggesting a neuroanatomical basis for the altered behavior. Overall, this study links alterations in developmental events to anxiety-like behaviors, which suggests that disruptions of FGF signaling may be a factor to consider in the neurodevelopmental origins of anxiety and affective disorders.Sources of Support: NIH R01 HD042634; R01 MH086539

3) **BXD recombinant inbred mice with low brain iron exhibit a reduced dopaminergic- mediated synaptic transmission in the CA1 region of the hippocampus**

AB Breton, JA Fox, MP Brownson, M.D. McEchron. From the WWAMI Department, University of Wyoming, Laramie, WY.

Iron deficiency (ID) is the most prevalent nutritional disorder in the world. Studies have shown that developmental ID is linked to learning impairments in humans. The BXD recombinant inbred mouse strains have been shown to have varying but consistent levels of brain iron. This could provide a useful genetic mouse model for examining the effects of ID on brain function, independent of nutritional intake. The BXD mouse strains are formed by litter mating of the inbred strains, C57BL/6J and DBA/2J. There are 28 known BXD recombinant inbred strains. The BXD 13 strain contains higher levels of brain iron when compared with the BXD 6 strain. Work has shown that the BXD 6 strain exhibits reduced levels of hippocampus-dependent learning compared to the BXD 13 strain. The present study used electrophysiological brain slice methods to examine dopaminergic-mediated synaptic efficacy in the hippocampus of BXD 13 and BXD 6 mice. Our previous work demonstrates that perinatal nutritional ID produces irreversible impairments in dopaminergic-dependent synaptic transmission in the hippocampus. Catecholamines such as norepinephrine and dopamine are known to play a pivotal role in memory consolidation. Studies have shown that catecholamine agonists produce a long lasting potentiation in synaptic transmission. Mice were maintained on a normal iron diet (90 ppm iron) through pregnancy and postnatal development. Hippocampal brain slices were prepared between postnatal day 26 and 30, and synaptic efficacy was measured in the CA1 region of the hippocampus by examining population spike amplitude. Slices were treated with the dopaminergic agonist SKF-38393 to induce modulatory increases in synaptic efficacy. Slices obtained from the high brain iron BXD 13 mice (n=10) exhibited a long-lasting increase in synaptic efficacy as the result of SKF-38393 perfusion. The low brain iron BXD 6 slices (n=10) showed little or no increase in synaptic efficacy as a result of SKF-38393 perfusion. These results demonstrate that genetic mediation of low brain iron may impair or reduce dopaminergic-dependent synaptic plasticity in the hippocampus.

Development

4) **Real-Time Nitric Oxide Release Measurements: Towards Understanding Cell Migration in Brain Tissue**

CM Bishop[1], MM Reynolds[1,2]. From the [1]Department of Chemistry and [2]School of Biomedical Engineering, Colorado State University, Fort Collins, CO.

Nitric oxide (NO) is involved in many biological pathways including cellular migration. In brain tissue, the NO concentration responsible for cell movement ranges from 0.38 to 1800 nM (1). As a part of a broader project focused on better understanding the NO kinetics involved in cell movement, we have developed a measurement method that allows for real-time and quantitative monitoring of gaseous NO in aqueous buffer. Key system components consist of a custom sample cell with a pH probe and multiple gas supply inputs, a flow-regulated CO2 delivery system, and a chemiluminescence detector. In this presentation, we will discuss the use of these components in a manner that allows for a) NO measurements in the physiological range and b) the ability to validate other NO measurement devices. The system development was based upon measuring the NO released from a well-established NO donor (MAHMA\NO) in aqueous buffer at 37 °C and pH of 7.40 (2). Modifications to the volume of the sample cell and flow rates of the gas delivery system allowed lower detection limits to be achieved while maintaining the reported NO-release kinetics. In separate studies, through the use of appropriate CO2 flow rates, precise control of the pH of tissue media was achieved. Taken together, the new system now enables measurements of NO dosages in a bench-top system that mimics the conditions of an in vitro tissue assay. In this way, better relationships between the dosages of NO donors used to actual NO delivered and eventually cell movement can be established.

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Wink, D. A.; Saavedra, J. E.; Keefer, L. K., Chemistry of the Diazeniumdiolates.
2. Kinetic and Mechanisms of Dissociation to Nitric Oxide in Aqueous Solution. Journal of American Chemical Society 2001, 123 (23), 5473-5481.

EXPERTISE: Chemiluminescence

5) **Adult amphetamine-induced dopamine release in the medial prefrontal cortex and nucleus accumbens following adolescent social defeat in rats.**

AR Burke, GL Forster, AM Novick, CL Roberts & MJ Watt. From the Neuroscience Group, Division of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, Vermillion, SD.

Final maturation of dopamine systems occurs in adolescence, and social stress during this period may increase vulnerability to addiction in adulthood. Male rats exposed to repeated social defeat in adolescence exhibit increased preference for amphetamine (AMP) cues in adulthood (P70). We chose to investigate the effects of social defeat in adolescence on adult AMP-elicited dopamine release in the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) based on our previous findings. Rats were exposed to repeated social defeat (once daily from P35 to P39), with controls placed in empty novel cages at matched times. All rats were then left undisturbed to mature into adulthood. In early adulthood (P56), acute AMP (1.0 mg/kg, i.p.) or saline injections were administered with locomotor activity observed for 90 min. Two days later, rats were anesthetized, microdialysis probes implanted into the mPFC and NAc core, and AMP-induced dopamine release was simultaneously measured in the mPFC and NAc

using HPLC with electrochemical detection. In a separate repeated AMP experiment, rats received AMP (1.0 mg/kg) or saline once per day for 5 days. On the 6th day AMP-induced locomotion was measured. On the 7th day of repeated AMP experience, AMP-induced dopamine release was measured as mentioned above. For rats that were exposed to adolescent defeat, there was a greater acute AMP-induced locomotion, and less AMP-induced dopamine release in the mPFC compared to AMP-receiving controls. After repeated AMP experience, rats defeated in adolescence exhibited AMP-induced locomotion that was no different from AMP-receiving controls. Furthermore, repeated AMP experience attenuated the low mPFC AMP-induced dopamine release that was observed following adolescent social defeat and acute AMP in adulthood. In the NAc core, AMP-induced dopamine release was unaltered by adolescent social defeat in the acute and repeated AMP experiments. These data suggest that adolescent defeat may cause a blunted mPFC dopamine response to AMP and an increased locomotion response to acute AMP in adulthood, which are both normalized by repeated AMP injections.

6) **Supplementation of the maternal diet with free sialic acid increases neurite growth and white matter maturation in the offspring.**

AE Garrison, MJ Weiser, JP Zimmer, CM Butt. From Discovery Neuroscience, DSM Nutritional Products, Boulder, CO.

The level of N-acetylneuraminic acid (aka sialic acid; SA) in mother's milk is generally higher than that found in baby formula, but the function of SA in pre-, peri-, and postnatal development is poorly understood. Previous work has suggested that direct administration of SA to developing animals is associated with better cognitive performance later in life. However, it is unknown whether supplementation of the maternal diet with SA confers any measurable changes in the neurodevelopment of the offspring. We sought to address this question by evaluating neurite extension and myelin basic protein (MBP) expression in rat brain tissue derived from offspring whose mothers had been fed SA during gestation or during lactation.
Neurite extension was then assessed in primary hippocampal neurons of the offspring, and MBP expression was measured in the forebrain, cerebellum, and hindbrain of the offspring by immunohistochemistry and western blot. Supplementation of the gestational maternal diet with SA was associated with greater hippocampal neurite extension and increased thickness of MBP expression in the corpus callosum, lateral olfactory tract, motor pyramids, and medial lemniscus. Similarly, supplementation of the maternal diet with SA during lactation was associated with increased thickness of MBP expression in the corpus callosum and lateral olfactory tract, but not in the motor pyramids or medial lemniscus. In addition, lactational supplementation was associated with overall MBP expression in the cerebellum as measured by western blot. The current findings suggest that a mother's dietary SA may enhance brain development in her offspring, but such benefits may depend on the timing of optimal SA availability and differential sensitivities of developing brain regions.
EXPERTISE: IHC, ICC, in situ, qRT-PCR, tissue culture, behavior

7) **Tbx5 function is dispensable for cell proliferation in zebrafish development.**

LE Parrie, EM Renfrew, MA DeMiranda, DM Garrity. From the Biology Department, Colorado State University, Fort Collins, CO.

Mutations in the T-box transcription factor TBX5 result in malformations of embryonic cardiac and forelimb structures. Tbx5 heterozygosity in humans leads to a condition known as Holt-Oram Syndrome (HOS). Homozygous mutation of zebrafish tbx5a similarly affects both heart and forelimb formation; however, cardiac defects are lethal

and forelimb structures (pectoral fins) are completely absent. Previous Tbx5 overexpression studies in chick and mouse demonstrated that Tbx5 provides a growth arrest signal that limits cardiomyocyte proliferation during chamber morphogenesis stages (Hatcher et al., 2001). In the converse experiment we find that a tbx5a loss-of-function mutation in zebrafish, named heartstrings (hst), did not lead to increased cardiomyocyte number, and ultimately had no net effect on cell proliferation of cardiomyocytes at chamber morphogenesis stages at all. We also demonstrate that knockdown of tbx5b (a tbx5a paralog in zebrafish) does not alter cardiomyocyte proliferation. Because cardiomyocyte proliferation does not appear to contribute to the hst phenotype, we hypothesize that inability of the hst heart tube to loop may result from the deficiencies in overall cardiomyocyte size and elongation of atrial inner curvature cells we have observed. Finally, we are able to conclusively determine that two tbx5a mutations, including hst, encode null alleles via Western Blot analysis.
EXPERTISE: in sity hybridization, real time qPCR, microinjection, cloning, transgenesis, Western Blot

8) **The conserved P-body component HPat/Pat1 regulates synaptic structure at the larval Drosophila neuromuscular junction.**

SJ Pradhan[1], K Nesler[1], LM Rozeboom[1], Y Kato[2], A Nakamura[2], M Ramaswami[3], SA Barbee[1]. [1]From the Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver CO; [2]Laboratory for Germline Development, RIKEN Center for Developmental Biology, Kobe, Japan; [3]Smurfit Institute of Genetics and TCIN, Lloyd Building, Trinity College Dublin, Dublin, Ireland.

Neurons contain a vast array of RNPs (ribonucleoprotein particles), also called RNA granules, that traffic repressed mRNAs in dendrites. A subset of RNA granule, in Drosophila melanogaster contains proteins associated with mRNA processing bodies, or “P bodies”. P bodies are cytoplasmic RNP particles linked to both mRNA decay and translational repression pathways. An actively translating mRNA may exit translation upon receiving a signal and assemble into a RNP. Translation regulation locally at the synapse is important in the control of both synapse structure and function. Drosophila HPat is a conserved P body component and is required for both mRNA deadenylation and decapping. Here, we show that HPat, regulates synaptic structure at the Drosophila larval neuromuscular junction (NMJ) both during development and following acute synaptic stimulation. We also demonstrate that HPat is expressed in the CNS and localizes to FMRP-containing neuronal granules. Finally, we show that HPat functions on the pre-synaptic side of the synapse and interacts genetically with conserved components of the microRNA pathway (Argonaute 1) to regulate synapse structure.
EXPERTISE: Immunohistochemistry, In situ hbridization, neuron culture

9) **The miRNA pathway controls rapid activity-dependant changes in synapse structure at the Drosophila neuromuscular junction.**

KR Nesler, RI Sand, BA Symmes, ML Ritz, SA Barbee. From the Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver, CO.

It is well established that some forms of long-term memory (LTM) depend on local protein synthesis at the synapse triggered by synaptic activity. A growing body of evidence implicates the microRNA (miRNA) pathway as an important mediator in these processes. However, while great advances have been made in the field, little has been done to identify and characterize the specific activity-dependent neuronal miRNAs implicated in the control of synaptic plasticity, a cellular correlate of LTM. Using an acute spaced training high potassium stimulation paradigm (Ataman et al., 2008) we have observed a rapid, activity-dependant increase in new synaptic growth at the

Drosophila neuromuscular junction (NMJ). In a targeted screen using real-time quantitative PCR (RT-qPCR) we have identified five miRNAs that are significantly down-regulated in response to synaptic activity in the Drosophila third instar larval CNS. These results indicate a specific reduction in miRNA expression following neuronal activity stimulated by the high potassium spaced training paradigm. We are validating these miRNAs predicted to modulate synaptic structure as activity-dependent through experiments where we drive the over-expression of each individual miRNA in presynaptic motor neurons and examine the phenotypic changes at the NMJ in response to the spaced training paradigm. We hypothesize that the miRNAs that are significantly down-regulated in response to activity and are validated as activity-dependent will control the expression of genes involved in the control of synaptogenesis, NMJ structure and/or function, as well as learning and memory. We are using a luciferase-based assay to validate these predicted mRNA/miRNA interactions in Drosophila cell-culture. The current results of this screen and validation experiments will be presented in detail.

10) **Gonadal steroid influences on the development of perinatal sex differences in the preoptic area.**

BT Searcy, P Kumar, MS Stratton, SA Tobet. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.

The preoptic area/anterior hypothalamus (POA/AH) plays a key role in the regulation of sex specific behaviors. Correspondingly, sex differences in the density and location of neurons in the POA/AH have been identified. One mechanism through which these differences may develop is through differential neuron migration. Estradiol signaling has been shown to rapidly alter cell movements in the embryonic mouse POA/AH, and could therefore impact ultimate neuron positioning. Here we characterize changes in movement of live neurons in organotypic POA/AH slices to the selective estrogen receptor agonists propylpyrazole triol (PPT), S-diarylpropionitrile (S-DPN) and STX. PPT and S-DPN are selective estrogen receptor α and β agonists, respectively. STX mimics rapid estradiol effects on POMC neurons. S-DPN induced a rapid reduction in the rate of cell movement in the POA/AH, whereas STX exposure had no detectable effect. We have also investigated the role of perinatal gonadal steroids on development of sex differences in calbindin-immunoreactivity in the POA-AH of the mouse. Here we report characterization of the impact of perinatal estradiol-benzoate, testosterone-propionate, and dihydrotestosterone-propionate exposure on postnatal day 10 patterns of calbindin-immunoreactivity. One mechanism through which steroids may rapidly transduce signals to perinatal neurons is through induction of nitric oxide production. Neuronal nitric oxide synthase (nNOS) has been characterized with a distinct cellular distribution in POA/AH. To investigate the impact of nNOS on neuron location, the distribution of immunoreactive (ir)-calbindin was compared between nNOS-knockout (KN2) and wild type male mice on postnatal day 0. The spread of ir-calbindin was 50% greater in wild-type animals than nNOS knockout mice ($p < 0.01$.The current findings indicate a potential role for both ERβ and NO in determining cell positions within the developing POA.

11) **Mechanical Properties of Hyaluronic Acid-Poly(Ethylene Glycol)-based Hydrogels for the Development a Synthetic Brain Extracellular Matrix.**

ER Aurand, KB Bjugstad. From the Neuroscience Program and Department of Pediatrics, University of Colorado – Anschutz Medical Campus, Aurora, CO.

A major goal of tissue engineering is to recreate the extracellular matrix (ECM) of tissues, including brain tissue. To be effective, a synthetic ECM must mimic the

chemical, mechanical, and physical properties of the brain tissue that support the survival of replacement cells. Despite the chemical simplicity of most synthetic ECM materials, the mechanical and physical properties have substantial impact on the engrafted cells, especially neural progenitor cells (NPC). To date, no such materials have been thoroughly characterized in regards to these properties and the outcomes they produce in a manner which can be easily replicated. Using hyaluronic acid (HA) and poly(ethylene glycol) (PEG), we are developing simple polymer hydrogels to be used in neural tissue engineering and repair as a synthetic ECM. Hydrogel compositions containing 0.2% to 1.0%wt HA and 0.6% to 3%wt PEG were used to create 25 different HA-PEG hydrogel formulations. The compressive modulus of each formulation was measured using unconfined compression at a rate of 200μm/min to an end strain of 40%. We report here that the 25 formulations demonstrate a range of mechanical properties which encompass the material properties hypothesized to be successful for the growth and maturation of NPC and which are consistent to those measured in brain tissue. The compressive moduli range from 0.3 to 60kPa for the HA-PEG hydrogels. Parallel measures of brain tissue indicate a mean compressive modulus of 3.5kPa for mouse tissue and 9kPa for rat tissue. These results suggest that within the hydrogel compositions we have designed, a number of formulations will support NPC and may be further developed to mimic the brain ECM in other ways.

12) Vasculature within the paraventricular nucleus of the hypothalamus: Development, location and GABA signaling.

KA Frahm, MJ Schow, Q Zhang, CM Eitel, SA Tobet. From the Program of Cellular and Molecular Biology, the College of Veterinary Medicine and Biomedical Sciences, and the School of Biomedical Engineering, Colorado State University, Fort Collins, CO.

The paraventricular nucleus of the hypothalamus (PVN) is a noticeably vascularly-dense nuclear group that plays important roles in regulating homeostatic function. Within and around the PVN are several subpopulations of neurons expressing gamma-aminobutyric acid (GABA) receptors that impact the development of the PVN based on loss of function studies. In rats striking angiogenic changes were reported in the PVN over the first 2 postnatal weeks that involved decreases right after birth followed by increases in mid and caudal regions. To characterize the postnatal angiogenic process in mice, blood vessels were visualized by immunolabeling for platelet endothelial cell adhesion molecule (PECAM) in 50μm serial sections. Blood vessel density was determined quantitatively at P8, 20 and 50 in rostral, mid and caudal regions of the PVN in males and females. Changes relative to disruption of the R1 subunit of the GABAB receptor (knockout or KO) were evaluated at P8 and P20. Density was estimated using blood vessel length and a crude measure of complexity that included blood vessel branching and bending. There was an age difference in total blood vessel density with P20 and 50 mice having greater densities for the rostral, mid and caudal regions compared to P8 ($p < 0.05$). At P20, there was a strikingly greater blood vessel density within the mid region compared to rostral and caudal regions ($p < 0.05$). From P20 to adult, there was a greater increase in blood vessel density within the caudal region compared to the rostral and mid regions ($p < 0.05$). There were no differences on P8 due to sex, region, or GABAB R1 KO. However, there was a significant 20% decrease in the mid region in GABABR1 KO versus wild type at P20 ($p < 0.05$). These findings suggest that the loss of GABAB signaling may lead to a late developing defect in PVN angiogenesis. In summary, the data show that in the PVN of mice there is a progressive postnatal angiogenic period that begins rostrally and progresses more caudally. The loss of vascularity with defective GABAB signaling suggests that neurovascular relationships in

the PVN may be an important locus for understanding disorders of the hypothalamic-pituitary-adrenal axis with impact for psychiatric disorders.
EXPERTISE: Immunocytochemistry, in situ hybridization, real time qPCR, microscopy, computer data analysis.

13) **Embryonic GABA-B receptor blockade alters adult hypothalamic structure, and anxiety- and depression-like behaviors in mice**

M Stratton, T Budefeld, G Majdic, SA Tobet. From the Program of Cellular and Molecular Biology and the College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

The paraventricular nucleus of the hypothalamus (PVN) regulates the autonomic nervous system and neuroendocrine stress responses. Data suggest that GABA, acting through GABAB receptors, is important for the development of the PVN. We have shown that mice lacking functional GABAB receptors have sex specific alterations in PVN cell placement and protein expression. The current experiments used 2-Hydroxy-saclofen (2HS) to determine whether embryonic receptor antagonism alters the structure of the PVN (produces a phenocopy of the receptor knockout mouse) and behaviors that may depend on PVN function. Time mated C57BL/6 dams were injected with PBS vehicle or 1mg/kg of 2HS daily from embryonic days (E) 10-17. At 50 - 60 days of age, animals were subjected to elevated plus maze and forced swim tests. In males, serum was collected the day before the forced swim test, 15 minutes after the forced swim test and at harvest, 3 hours after the test. Immunohistochemical analyses were conducted on perfusion fixed tissue to assay the placement of immunoreactive estrogen receptor α and neuronal nitric oxide synthase (nNOS) in the PVN. Adult female mice that were subjected to embryonic GABAB receptor blockade showed a lateral shift in ER-alpha immunoreactivity from the PVN to the lateral hypothalamic/perifornical area ($F(8, 88) = 2.33$, $P < 0.05$). Immunoreactive nNOS also appeared to spread more laterally in male and female 2HS exposed mice compared to vehicle controls. Male 2HS treated mice showed a strong trend toward increased anxiety-like behavior in the elevated plus maze (mean time(s) closed arm: 184 +/- 8.1, Veh N=6 vs 207 +/- 11.5, 2HS N=8, p=0.07 T-test) and increased depressive like behavior in the forced swim test (mean time(s) immobile: 178 +/- 16.9, Veh N=8 vs 225 +/- 15.0, 2HS N=10 p<0.05 T-test). Pharmacological blockade of the GABAB receptor copied the neuroanatomical phenotype seen in the GABAB receptor knockout mouse. The sex dependent nature of these findings highlights the complexities of neuronal development and the regulation of complex behaviors. It is possible, if not likely, that neurologic disorders arise via different mechanisms with roots in different brain regions in males and females.

14) **Connexin 35 in zebrafish spinal cord development**

TC Martin, AB Ribera. From the Department of Physiology and Biophysics, Neuroscience Program, Colorado Clinical and Translational Sciences Institute, University of Colorado Anschutz Medical Campus, Aurora, CO.

Vertebrate connexin proteins form the majority of gap junction channels allowing direct communication between coupled cells. In both neuronal and non-neuronal tissues, many connexin proteins display strong embryonic expression and are subsequently downregulated, which raises the possibility these proteins may play developmental roles. The involvement of gap junction proteins in neurodevelopmental processes has recently become evident, but the underlying mechanisms by which these proteins may mediate developmental processes remain poorly understood. This project aims to explore the role of a specific connexin (connexin 35, Cx35) in spinal cord development. We are using the zebrafish embryo to uncover the developmental processes requiring Cx35.

RNA expression data indicate cx35 expression begins at 50% epiboly by RT-PCR and is localized to the spinal cord with RNA in situ hybridization at 24 hpf. Expression of cx35 is preferentially ventral in the spinal cord across all stages studied. RNA in situ hybridization studies in transgenic lines indicate cx35 is present in secondary motor neurons but not primary motor neurons at 24 hpf. Additionally, initial transient transgenic reporter expression with a BAC construct containing the Cx35 upstream sequence suggests cx35 is expressed in secondary motor neurons and interneurons at 72 hpf. The results of preliminary Cx35 morpholino knockdown and mutant studies suggest a requirement for this connexin protein in development of a subset of spinal cord cells. The ultimate goal is to identify the mechanistic basis for this role of Cx35, by first identifying the specific cell types that express Cx35 across relevant developmental stages, and next, determining the networks of electrically coupled cells that depend on Cx35 function and how these networks change during development. The results of these studies will provide insights into developmental mechanisms that require Cx35.

Disorders of the Nervous System

15) **Bcl-2 is a novel interacting partner for the 2-oxoglutarate carrier and a key regulator of mitochondrial glutathione**.

HM Wilkins, K Marquardt, LH Lash, DA Linseman
From the Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver and VA Medical Center, Denver, CO

Despite making up only a minor fraction of the total cellular glutathione, recent studies indicate that the mitochondrial glutathione pool is essential for cell survival. Glutathione is synthesized exclusively in the cytoplasm and must be actively transported into mitochondria. Therefore, regulation of mitochondrial glutathione transport is a key factor in maintaining the antioxidant status of mitochondria. Bcl-2 resides in the outer mitochondrial membrane and displays an antioxidant-like function that has been linked experimentally to the regulation of cellular glutathione content. We have previously demonstrated a novel interaction between rBcl-2 and reduced glutathione (GSH), which was antagonized by either BH3 mimetics or a BH3-only protein, rBim. These previous findings prompted us to investigate if this novel Bcl-2/GSH interaction might play a role in regulating mitochondrial glutathione transport. Incubation of primary cultures of cerebellar granule neurons (CGNs) with the BH3 mimetic HA14-1 induced MOS and caused specific depletion of the mitochondrial glutathione pool. Bcl-2 was co-IP'd with GSH after chemical cross-linking in CGNs and this Bcl-2/GSH interaction was antagonized by preincubation with HA14-1. Moreover, both HA14-1 and rBim inhibited GSH transport into isolated rat brain mitochondria. We next examined if Bcl-2 associated with the 2-oxoglutarate carrier (OGC), an inner mitochondrial membrane protein known to transport glutathione in liver and kidney. After cotransfection of CHO cells, Bcl-2 was Co-IP'd with OGC and this novel interaction was significantly enhanced by glutathione monoethyl ester. Similarly, rBcl-2 interacted with rOGC in the presence of GSH. Bcl-2 and OGC cotransfection in CHO cells significantly increased the mitochondrial glutathione pool. Finally, the ability of Bcl-2 to protect CHO cells from apoptosis induced by hydrogen peroxide was significantly attenuated by the OGC inhibitor phenylsuccinate. Bcl-2 and OGC appear to act in a coordinated manner to increase the mitochondrial glutathione pool. We conclude that regulation of mitochondrial glutathione transport is a principal mechanism by which Bcl-2 suppresses MOS.

EXPERTISE: western blotting, immunocytochemistry, tissue culture

16) **Acute regulation of MCT1 function in cerebrovascular endothelial cells by cAMP dependent vesicular trafficking.**

JP Smith, AL Uhernik, BN Nuanez, KT Darcy, M Sneve, Z Liu, LR Drewes. From the Deparment of Biology at Colorado State University-Pueblo and the deparment of Biochemistry and Molecular Biology University of Minnesota Scool of Medicine Duluth.

Monocarboxylic acid transporter 1 (MCT1) is located in cerebral microvascular endothelial cells where it is the only known facilitator of lactic acid transport across the blood brain barrier. Recent evidence strongly suggests that normal development of the neurovascular unit (NVU) and rapid regulation of blood-brain transport of short chain carboxylates (lactate, pyruvate, ketones) by the brain endothelial MCT1, require specific signaling pathways involving the cAMP/protein kinase A-dependent signaling. In our study of rat brain endothelial cells, RBE4, short term treatment with cAMP analogs, such as 8-Br-cAMP, regulated MCT1 function either positively or negatively depending upon the post subculture recovery period; however, the mechanism for this bimodal effect is unknown. Previous kinetic studies showed that MCT1 regulation by cAMP analogs affected Vmax but not KM suggesting that cell surface and cytoplasmic trafficking may be part of the mechanism. To examine more closely this potential mechanism of MCT1 regulation, the plasma membrane surface of RBE4 cells was isolated by biotinylation or phosphoprotein binding and quantified by immunoblot detection. Brief treatment with cAMP caused rapid dephosphorylation of MCT1 and a decline in membrane localization. These results indicate that MCT1 transporter activity is regulated by its plasma membrane location and may be dependent on its phosphorylation state. Dual immunostaining of RBE4 cells, subcultured for either 3 or 24 hours, showed extensive MCT1-Rab5 co-localization at the plasma membrane and in cytoplasmic puncta consistent with early/sorting endosomes. The patterns of expression and co-localization changed with 8-Br-cAMP in both subculture groups. Taken together, our surface biotinylation and immunofluorescence results suggest that endosomal trafficking of MCT1 is a key part of its regulatory pathway.

17) **Nutraceuticals offer protection from neuronal apoptosis resulting from nitrosative and oxidative stress in rat cerebellar granule neurons.**

JJ Gray, TC Sutcliffe, AN Winter, NA Kelsey, DA Linseman. From the Department of Biological Sciences/Eleanor Roosevelt Institute, University of Denver; Res. Ser., DVAMC., Denver, CO.

Oxidative stress and nitrosative stress occur when reactive oxygen species (ROS) or reactive nitrogen species (RNS), respectively, overwhelm cellular antioxidant defenses. Ultimately, these free radicals cause severe damage to critical cellular proteins, lipids, and DNA, culminating in cell death via either apoptosis or necrosis. These adverse conditions play a central role in the neuronal cell death that underlies a number of neurodegenerative disorders, such as amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease. The key role of oxidative and nitrosative stress in neurodegeneration has prompted interest in the discovery of novel therapeutic agents that mitigate the damaging effects of ROS and RNS. Nutraceuticals (natural products) possessing intrinsic antioxidant properties represent readily available compounds that have either ROS or RNS scavenging activity. Here, we evaluated the protective effects of several nutraceutical antioxidants in primary cultures of cerebellar granule neurons (CGNs) exposed to oxidative or nitrosative stress. Specifically, we induced oxidative stress at the level of the mitochondria by incubating CGNs with HA14-1, a BH3 domain mimetic that inhibits Bcl-2 function. Nitrosative stress was evoked by incubation with the NO generating compound, sodium nitroprusside (SNP). The nutraceutical antioxidants tested included procyanidin B2 (found in high concentrations in cocoa) and the

anthocyanins, kuromanin and callistephin (found in large amounts in black rice and strawberries, respectively). Pre-incubation of CGNs with procyanidin B2 or either anthocyanin offered significant protection against oxidative stress produced through inhibition of Bcl-2 with HA14-1. On the other hand, while procyanidin B2 and kuromanin each significantly protected CGNs from SNP, callistephin had no measurable effect on death induced by this nitrosative stressor. Our studies highlight the prospective applications of using nutraceuticals as novel antioxidant therapies for neurodegeneration and suggest that various natural compounds may show substantial differences in their capacities to scavenge either ROS or RNS.
EXPERTISE: immunocytochemistry, tissue culture, western blotting, molecular biology

18) **Neuroprotective effects of supra-nutritional selenium in a mouse model of Huntington's disease.**

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Huntington's disease is a progressive neurodegenerative disease caused by a glutamine encoding expansion within exon-1 of the huntiingtin gene. Mutant huntingtin protein accumulates in neurons throughout the brain; however, degeneration is most marked within the neostriatum and neocortex. Currently, there are no neuroprotective therapies for human HD. We tested the effect of selenium supplementation in the N171-82Q mouse model of HD after finding that low brain selenium is a prominent feature of the human HD phenotypes. We delivered selenium in drinking water as sodium selenite. HD mice were assigned to treatment groups at 4 weeks and supplementation provided from 6 weeks. Mice were sacrificed at 14 weeks corresponding to late- stage disease. There was a significant protective effect on motor endurance as measured b y Rota-rod analysis in selenium-supplemented mice. Brain biochemical studies demonstrated partial reversal of three changes that occur in HD mice compared to wild-type litter-mate mice. Striatal DARPP32 protein levels were increased; this protein is decreased in HD brain due to transcriptional dysregulation. Cortical oxidized glutathione (GSSG) were elevated in HD controls but reverted to wild-type levels in selenium supplemented mice. Further, high dose selenium supplementation also resulted in decreased levels of mutant huntingtin protein aggregates and increased brain mass, as compared to the HD controls. In-toto, our study shows that selenium supplementation in HD mice fed a standard mouse chow provides protection at the level of behavior, brain biochemistry and preservation of brain mass. These findings suggest that similar protective effects might occur in human HD.

19) **A novel method to incorporate nerve growth factor onto a polypyrrole coated nanowire scaffold for potential nervous tissue engineering applications.**

SL Bechara, KC Popat. From the Department of Biomedical Engineering, Colorado State University, Fort Collins, CO.

We are currently investigating the effect of nerve growth factor coated surfaces in relation to neural stem cell proliferation and differentiation. Live and cytoskelleton stains have showed potential neuronal and glial differentiation. Current immunohistochemical studies are directed at further understanding the differentiation of the neural stem cells by investigating Beta Tubulin III and GFAP. An NGF elisa is being performed to investigate if and how NGF is released from the surface.

20) **Subcortical auditory dys-synchrony affects cortical maturation in children with Auditory Neuropathy Spectrum Disorder.**

GJ Cardon, A Sharma. From the Speech, Language, and Hearing Sciences Department, University of Colorado at Boulder, Boulder, CO.

Auditory Neuropathy Spectrum Disorder (ANSD) is a recently discovered disorder that affects approximately 10% of children with hearing loss. ANSD is characterized by intact cochlear outer hair cell function and a dys-synchronization of the auditory nerve. While diagnosis of ANSD is routine, clinicians currently have no way of gaining information that might assist them in making and verifying management decisions in this population. Though other clinical tools fail in this population, Cortical Auditory Evoked Potentials (CAEP) can be recorded in people with this condition. The P1 CAEP has been used to investigate the maturation of the human central auditory system in both normal hearing and hearing-impaired populations. Because P1 latencies vary as a function of age, they can be used to infer the maturational status of the auditory cortex. By employing CAEPs in 45 children with ANSD, we took part in a naturally occurring experiment regarding the effects of abnormal extrinsic stimulation on cortical development. We hypothesized that abnormalities in the pattern of subcortical neural activity (i.e., dys-synchrony) would affect central auditory maturation.Results showed that by using the P1, children with ANSD who used hearing aids could be divided into three groups: 1) children with normal P1 latency, amplitude and morphology; 2) children with normal P1 morphology, but delayed P1 latency and decreased P1 amplitude; 3) children with abnormal or absent P1 responses. In contrast, children with ANSD who used cochlear implants could only be categorized into groups #1 and #2. In addition, scores on a test of behavioral auditory skill development differed between the above groups, such that children with normal P1 responses scored higher than those in other groups. We conclude that the P1 is a reliable measure of cortical maturation in children with ANSD. It seems that the degree of dys-synchrony occurring at subcortical levels directly affects the development of the cortex. Treatment with hearing aids and cochlear implants, in turn, may influence auditory cortical maturation by improving subcortical synchrony to differing degrees. The P1 also appears to be a good predictor of behavioral outcome in children with ANSD.
EXPERTISE: Electrocencephalography (EEG), evoked potentials (esp. auditory evoked potentials), audiometric procedures

21) **Genetic and pharmacologic blockade of NF-kB prevents ongoing neuroinflammation in the MPTP model of Parkinson's disease.**

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Inflammatory activation of glia is implicated in the progressive loss of dopaminergic neurons in Parkinson’s Disease (PD). Suppression of neuroinflammation may prove useful in slowing continuous neuronal degeneration. In the present study we investigate the efficacy of nuclear factor kappa B (NF-kB) inhibition in attenuating progressive dopamine neuron loss. We established a model of neurodegeneration employing MPTP in conjunction with probenecid in transgenic NF-kB-EGFP reporter mice in which we observed a progressive reduction of TH positive neurons assessed by stereology. In addition, robust activation of astrocytes and microglia was observed that correlated with increased activation of NF-kB. Using this model, we assessed the efficacy of novel para-substituted diindolylmethane (cDIM) compounds in attenuating continual neuron loss in vivo. cDIM5 (1,1-bis(3’-indolyl)-1-(p-methoxy)- methane), which induces downregulation of prototypic neuroinflammatory gene NOS2 in primary astrocyte cultures, was given via

oral gavage (50 mg/kg) once daily to mice following 7 days of MPTP treatment. Stereological assessment revealed a significant attenuation of dopamine neuron loss in animals treated with cDIM5, as well as decreased glial activation. This process was repeated using cDIM12 (1,1-bis(3'-indolyl)-1-(p-chlorophenyl)- methane), a Nurr1 agonist, that also attenuated dopamine neuron loss. Finally, mice deficient in astrocytic IKKβ were treated using the progressive MPTP and probenecid model and these animals displayed significantly less dopamine neuron loss than their wild type counterparts given the same treatment. These results suggest that NF-kB is an important pathway mediating neuroinflammatory activation of astrocytes leading to loss of dopamine neurons and that interdicting this pathway may be a viable therapeutic option for slowing the progression of PD.
EXPERTISE: Immunofluoresence, stereology, tissue culture, animal behavior, immunoblot, viral transfection, qRT-PCR, live-cell imaging.

22) **Effects of adolescent social isolation and acute anxiogenic drug treatment during adulthood on tph2 mRNA expression in female rats**

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Early life stressors are thought to increase an individual's susceptibility to mental health disorders, including anxiety disorders and depression, later in life. This increased susceptibility to mood disorders is particularly relevant for females, as they are more than twice as likely as males to be diagnosed with major depression. One way early life stress may increase later susceptibility is by decreasing an individual's resilience to stressors later in life. The mechanisms of this increased susceptibility are still largely unknown, although they are thought to involve a dysregulation of the brainstem serotonergic system. In this study we investigated the
effects of adolescent social isolation (from postnatal day P21 to P42) on the expression of tryptophan hydroxylase 2 (tph2) mRNA expression in female rats using in situ hybridization. Furthermore, we tested the effects of an acute challenge with the anxiogenic drug FG-7142 (a partial inverse agonist at the benzodiazepine allosteric site on the GABA-A receptor) in isolation-reared rats and group-reared controls. We predicted that social isolation during early-life would result in increased tph2 expression under baseline conditions and that FG-7142 would further increase tph2 in social isolates, but not group-reared rats. However, we found that FG-7142 decreased tph2 in both isolates and group-reared rats, while social isolation by itself had no effect. These results indicate a possible novel mechanism whereby FG-7142 affects tph2 gene expression.

23) **Integrative physiology of antidepressant drug action**

KF Dady[1], MW Hale[1,2], JL Lukkes[1], KJ Kelly[1], CL Raison[3] CL, CA Lowry[1]. From the [1]Department of Integrative Physiology and Center for Neuroscience, University of Colorado Boulder, Boulder, CO; [2]School of Psychological Science, La Trobe University, Melbourne, Australia; [3]University of Arizona Medical Center, Tucson, AZ.

Antidepressants of diverse pharmacological profiles induce sweating as a clinical side effect, suggesting that the pathophysiology of depression as well as antidepressant drug action may involve interactions with thermoregulatory pathways. To test the hypothesis that these two pathways are interlinked, we used an acute subthreshold (subthreshold for induction of antidepressant-like behavior) injection of the SSRI citalopram paired with exposure to warm ambient temperature. We found that citalopram, by itself, induced hyperthermia that was comparable to that induced

by exposure to increased ambient temperature (37 degrees C) for 85 min. Neither citalopram by itself, nor exposure to increased ambient temperature induced antidepressant-like effects in the forced swim test. However, when rats were both treated with citalopram and exposed to elevated ambient temperature, they experienced an exaggerated hyperthermia, and responded with antidepressant-like behavior (increased swimming) in the forced swim test. In addition, the core body temperature immediately prior to the forced swim test predicted the amount of antidepressant-like behavior ($p < 0.00008$). These data and other data from our lab provide a rationale for novel therapeutic strategies for the treatment of affective disorders, including development of novel antidepressant drugs.

Neural Excitability, Synapse and Glia

24) **Regulation of GABA and Glutamate Release from Proopiomelanocortin Neuron Terminals in Intact Hypothalamic Networks**

MS Dicken, RE Tooker, ST Hentges. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.

Peptides produced in hypothalamic proopiomelanocortin (POMC) neurons are clearly important for the regulation of energy balance. However, recent studies indicate that POMC neurons can also release classical amino acid (AA) transmitters when these neurons are maintained in primary cell cultures. Since neurons in culture may not retain their true in vivo phenotype, the present study was designed to determine if POMC neurons release AA transmitters from intact neuronal networks. The light-activated cation channel channelrhodopsin-2 (ChR2) was selectively expressed in POMC neurons in vivo. Whole-cell electrophysiologic recordings were then made in brain slices containing POMC-ChR2 neurons. Brief pulses of blue light depolarized POMC-ChR2 neurons and induced the release of GABA and glutamate onto unidentified neurons within the arcuate nucleus (ARC), as well as onto other POMC neurons. Opioid and GABAB receptor agonists readily inhibited AA transmitter release from POMC neuron terminals. Altogether, the data show that in addition to peptide transmitters, POMC neurons can release the AA transmitters GABA and glutamate, and that the release of these transmitters can be dynamically regulated. Further, the results indicate that POMC neurons terminate heavily within the ARC innervating both POMC and non-POMC neurons. The physiologic consequence of AA transmitter release from POMC remains to be determined but may be significant given the recently described homeostatic functions for AA transmitters released from other hypothalamic neurons.
EXPERTISE: electrophysiological paradigms, stereotaxic injection, optogenetics

25) **ATP-dependent calcium signaling in striatal astrocytes is acutely sensitive to inhibition by structurally diverse cationic neurotoxicants**

K Streifel, A Gonzales, B Trout, L Maxwell, B Mohl, S Earley, R Tjalkens. From the Department of Environmental and Radiological Health Sciences and Biomedical Sciences, Colorado State University, Fort Collins, CO.

The basal ganglia are group of midbrain nuclei important for control of motor function that are highly sensitive to damage from oxidative stress, inflammation, and environmental neurotoxicants. Here we propose that inhibition of transmitter-evoked calcium signaling in astrocytes may contribute to this sensitivity because this pathway modulates diverse trophic functions in the CNS, including metabolism, synaptic activity, and regional cerebral blood flow (rCBF). To examine mechanisms underlying alterations in Ca2+ signaling in astrocytes, we postulated that several structurally diverse

neurotoxicants of the basal midbrain, all of which are cationic, would inhibit transmitter-induced calcium (Ca2+) signaling in cultured astrocytes: MPP+, the active metabolite of the model parkinsonian neurotoxicant, 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP); Paraquat (PQ); 6-Hydroxydopamine (6-OHDA); and Manganese (Mn2+). Using Ca2+ imaging in primary cultured striatal astrocytes, we investigated the effect of acute treatment with each neurotoxicant on agonist-induced intracellular Ca2+ transients. We observed a dose-dependent decrease in ATP-induced intracellular Ca2+ transients and mechanically stimulated Ca2+ waves in cultured astrocytes following acute application of MPP+, 6-OHDA, PQ and Mn2+. These compounds also acutely inhibited OAG-induced intracellular Ca2+ transients, suggesting that a receptor-operated cation channel such as the transient receptor potential (TRP) channel might be targeted. The TRPC3 channel antagonist, Pyr3, blocked OAG-induced intracellular Ca2+ transients similarly to MPP+, PQ, and Mn2+, but not 6-OHDA. Moreover, acute application of MPP+ also inhibited TRPC3-like currents, as determined by whole cell patch clamp experiments. These findings indicate that endogenous and exogenous chemicals that are structurally diverse but that have cationic properties inhibit ATP-induced Ca2+ signaling in astrocytes and may therefore share a common mechanism of neurotoxicity in their capacity to disrupt trophic functions reliant on this signaling phenomenon.
EXPERTISE: Tissue culture, real time fluorescence microscopy, mouse colonies, immunocytochemistry

26) **Proopiomelanocortin neurons in the arcuate nucleus have inhibitory and excitatory subpopulations.**

BC Jarvie, ST Hentges. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.

Hypothalamic proopiomelanocortin (POMC) neurons have traditionally been defined by their ability to make multiple peptides implicated in feeding behavior and reward. However, there is also evidence that subpopulations of these neurons release the inhibitory and excitatory amino acid (AA) transmitters, GABA and glutamate. In the present study fluorescent in situ hybridization and immunohistochemistry was used to describe POMC neurons with respect to their AA transmitter phenotype by labeling mRNA for a variety of markers which are indicative of GABA release, GAD67, GAD65, and vGAT, or glutamate release, vGLUT2. This method was compared to the use of transgenic GAD67-GFP and vGAT-GFP animals. Only about 7% of POMC neurons expressed vGLUT2, with a higher probability of occurrence in the retrochiasmatic or rostral-most arcuate nucleus. Roughly half of the vGLUT2 expressing POMC cells also expressed GAD65, although GAD65 was found in 41% of POMC neurons. Interestingly, despite multiple reports of GABA release from POMC neurons, the presence of the vesicular transporter responsible for packaging GABA, vGAT, was not detected. Subpopulations of POMC neurons can release inhibitory and/or excitatory AA onto downstream target sites which may mediate their actions in food intake and reward, allowing for a fast-acting mechanism of action rather than the slower release of POMC peptides.

27) **Hydrogen peroxide mediates oxidative stimulation of L-type calcium channels in arterial smooth muscle.**

NL Chaplin, GC Amberg. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.

Changes in calcium and redox homeostasis influence multiple cellular processes. Dysregulation of these signaling modalities is associated with pathology, especially in the cardiovascular system. While calcium and oxidant signaling are often considered to

be functionally intertwined, experimental evidence in support of this supposition is sparse. To address this important issue we tested the hypothesis that the ubiquitous reactive oxygen molecule hydrogen peroxide mediates oxidant-dependent stimulation of rat cerebral arterial smooth muscle L-type calcium channels. Using a combinatorial approach including conventional electrophysiology and total internal reflection fluorescence imaging, we found that application of exogenous hydrogen peroxide to isolated arterial smooth muscle cells increased localized calcium influx through L-type calcium channels. Similarly, oxidant-dependent stimulation of L-type calcium channels by the vasoconstrictor angiotensin II was abolished by intracellular application of catalase. Catalase also prevented angiotensin II from increasing localized subplasmalemmal oxidation previously associated with colocalized calcium influx through L-type channels. Furthermore, catalase attenuated the contractile response of intact cerebral arterial segments to angiotensin II. In contrast, enhanced dismutation of superoxide to hydrogen peroxide with superoxide dismutase had no effect on angiotensin-dependent stimulation of L-type calcium channels. We conclude that hydrogen peroxide is necessary for oxidant-dependent regulation of rat arterial smooth muscle L-type calcium channels. These data also support the emerging concept of hydrogen peroxide as an important oxidant second messenger in multiple cell types with a diverse array of biological functions.
EXPERTISE: eletrophysiology, fluorescence microscopy

28) **Site-directed metabolic biotinylation of AMPA receptors may perturb protein-protein interactions with TARPS.**

A Dudek, LM Stone-Roy, KM Partin. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO

AMPA receptors are glutamate-gated ion channels that mediate fast excitatory synaptic responses. AMPA receptor expression is regulated by stargazin, a transmembrane AMPA receptor regulatory protein (TARP). The goal of the present study is to probe the quarternary structure of AMPA receptor-TARP complexes at the plasma membrane. We hypothesized that biotin acceptor domains (BADs), expressed as cassettes of 32 amino acids that include the 17 amino acid BAD motif and a flexible glycine/alanine linker, could be inserted at putative sites of interaction in rat GluA2, and that subsequent biotinylation of these sites might sterically block functional interactions between GluA2 and stargazin (gamma-2) protein. The sites at which BADs were inserted (GluA2-BAD mutants) include parts of the receptor that lie directly above, below and within the transmembrane helices. GluA2-BAD mutants were transiently transfected into HEK 293 cells with and without stargazin and viewed using confocal fluorescence microscopy. We found that GluA2-BAD mutants were efficiently expressed in HEK 293 cells, suggesting that the insertions alone did not significantly impair receptor trafficking or function. Specifically, expression of GluA2-BAD MKV (an insertion below the M4 transmembrane domain) led to predominantly intracellular localization of the protein, similar to the wild type receptor, and co-expression of GluA2-BAD MKV with stargazin resulted in efficient trafficking of the mutant receptor to the plasma membrane, similar to wild type receptor. Our results demonstrate that insertion of the biotin acceptor domain itself at this site is not enough to prevent association with stargazin. Ongoing experiments will include a functional analysis of GluA2-BAD MKV using patch clamp electrophysiology, as well as the testing of other GluA2-BAD mutants that we have constructed. These experiments will lead to a better understanding of how the activity-dependent association of GluA2 with auxiliary proteins impact AMPA receptor synaptic physiology.
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29) **Activity-dependent retrograde signaling lowers voltage-gated calcium current threshold in retinal bipolar cells**

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Activity-dependent modulation of presynaptic voltage-gated calcium currents (VGCCs) can mediate synaptic plasticity. For example, high synaptic glutamate levels can activate metabotropic glutamate receptors (mGluRs) and trigger endocannabinoid release from postsynaptic neurons. In turn, retrograde endocannabinoid inhibition of presynaptic VGCCs reduces glutamate release, and synatic efficacy. Visual signals in the retina must pass through bipolar cells (BCs) on their way to the brain, because BCs form the sole direct excitatory glutamatergic connection between photoreceptors and ganglion cells (GCs), whose axons form the optic nerve. VGCCs of BCs mediate the kinetics of glutamate release to GCs, thereby the latency of first action potential sent to the brain, which is critical for coding visual scenes. The objective of the present study was to elucidate whether VGCCs of BCs are subject to actvity-dependent retrograde modulation.

Results: Strong depolarization of the axon terminal of identified, Mb type BCs in retinal slice preparation resulted in ~5 mV leftward shift of the VGCC's half-activation potential. The shift was not seen using Ba2+ as charge carrier of VGCC or in the presence of 10 mM intracellular BAPTA. Furthermore, in slice, CB1 endocanabinoid receptor block could not prevent the strong depolarization induced shift in VGCC activation, whereas an mGluR1 agonist could mimick it in the presence 10 mM intracellular BAPTA in the Mb terminal. Importantly, strong depolarization did not alter VGCC activation in cultured, solitary Mb BCs.

Conclusion: VGCCs of Mb BCs are subject to use-dependent modulation, which appears to be mediated by a synaptic pathway, triggered by robust presynaptic glutamate release. However, it is not the classical mGluR1-CB1 retrograde signaling pathway. The process likely boosts glutamate release from Mb BCs, particularly in response to weak inputs. Noteworthy, that some GC's response to weak stimulation increases following a robust stimulation (sensitization) while other GC's response decreases (adaptation). The novel mechanism described here might underlie the sensitization of GC responses, which is thought to prevent visual information loss due to adaptation.

EXPERTISE: electrophysiology, tissue culture

30) **Kv2.1 non-conducting state is dependent on cell-surface density, independent of cell-surface localization, and present endogenously in cultured hippocampal neurons.**

PD Fox[1,2], RL Loftus[1], E Deutsch[1], MM Tamkun[1,2]. From the [1]Department of Biomedical Sciences, [2]Program in Molecular and Cellular & Integrative Neurosciences, Colorado State University, Fort Collins, CO, USA

Expression of eGFP-Kv2.1 reveals two distinct populations of channels expressed in equal proportions, those retained within clusters, which are incapable of conducting K+, and non-clustered channels freely diffusing in the membrane. We hypothesized that all whole-cell current is derived from non-clustered channels. The goals of our present work were to 1) use single channel fluorescence to compare eGFP-Kv2.1 channel number to whole-cell current in HEK cells and 2) compare levels of endogenous Kv2.1 and Kv current in cultured hippocampal neurons. Since half of eGFP-Kv2.1 is localized to clusters and is non-conducting, we would expect the proportion of expressed to conducting channels (E to C proportion) to be roughly 2. Instead this proportion was 4.303±0.815 (n=21) meaning a portion of non-clustered channels are also non-conducting. The E to C proportion ranged from 0.956 to 13.17 with a strong positive

correlation to the density of channel expression. Furthermore, the E to C proportion for a Kv2.1 mutant which does not localize to clusters was 1.83±0.19 (n=10) indicating roughly half of non-clustered Kv2.1 is also non-conducting. This is contrasted by the E to C proportion of Kv1.4 which is 1.08±0.12 (n=9), a channel for which there is no known non-conducting population. Endogenous Kv2.1 expression in cultured E18 hippocampal neurons at 14 days in vitro (DIV) was determined by immunocytochemistry, standardized to eGFP-Kv2.1 in HEK cells, and compared to total delayed-rectifier current (IkDR). Average immunofluorescence corresponded to 96,578 ±11,317 channels (n = 18) in DIV 14 neurons and IkDR in these neurons averaged 13,435 ±1,558pA at +60mV (n = 19). Since Kv2.1 mediates roughly 50% of IkDR this corresponds to 11,646 ±1,351 Kv2.1 channels conducting K+ giving us an E to C proportion of 8.112 ±0.95 (n=19). Thus we find that neurons endogenously express a large population of non-conducting Kv2.1 channels, which are localized both within and outside clusters, and in this manner endogenous Kv2.1 behaves similarly to eGFP-Kv2.1 in HEK cells, which are regulated in a density dependent manner.
EXPERTISE: Live-cell imaging, Electrophysiology

31) **Design and Profiling of a series of AMPA receptor modulators.**

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Glutamate is the primary excitatory neurotransmitter in the mammalian CNS. One of its targets, the AMPA family of ion channels, is responsible for mediating neural processes involved in learning and memory. Further, it has been shown that these processes are enhanced by modulatory drugs acting directly on AMPA receptors. Due to these positive effects, AMPA receptors and their allosteric modulators present key targets of study in the treatment for cognitive disorders. To expedite development of positive AMPA receptor modulators, a unique structure-based approach to drug design (SBDD) is being used to rapidly screen compounds for modulatory effects and optimize them for use as drug based treatments. So far SBDD has been successful at creating and testing drugs with positive in vivo results, but the method of action for these drugs on their pre-identified targets is not well understood. In the present study, we focused our efforts on the yet under-utilized benefit of SBDD to gain a better understanding of AMPA receptor mechanisms of gating. Using a fast-exchange perfusion system to simulate synaptic events, we compared the effects of two well documented allosteric modulators (cyclothiazide and CX614) to one of Merck's new chemotypes, JAMI 1001A ((2-(2-4-(hydroxymethyl)-3-(trifluoromethyl)-1H-pyrazol-1- yl)acetamindo)-4,5,6,7 tetrahydrobenzo[b]thiophene-3-carboxamide). Outside-out patches excised from HEK 293 cells transiently transfected with the AMPA receptor GluA2 were exposed to glutamate agonist with or without modulator. The JAMI 1001A compound was found to be more efficacious on GluA2 than its predecessors, slowing the onset of receptor deactivation by more than 2-fold. In addition, JAMI 1001A significantly modulated desensitization, resulting in a robust steady-state current in the continued presence of glutamate. Computational modeling suggests that JAMI 1001A may provide new insight into AMPA receptor channel gating. The present study demonstrates a two- fold benefit of SBDD: it confirms SBDD as an effective means for drug development, and further demonstrates SBDD as a useful tool in understanding drug and receptor mechanisms of action.
EXPERTISE: Electrophysiology, tissue culture, site-directed mutagenesis

32) **Direct CAMP binding and PKA phosphorylation share a common gating mechanism in HCN4 channels**

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Sympathetic regulation of HCN4 channels can occur via two cAMP-dependent pathways: either direct binding of cAMP to a cyclic nucleotide binding domain, or PKA phosphorylation of the distal C terminus. Here, we have investigated the energetic interactions between these two modulatory mechanisms. cDNA encoding wildtype or mutant mouse HCN4 channels was expressed in HEK293 cells, and the voltage-dependence of channel activation was determined in whole cell voltage clamp experiments. Intracellular dialysis of either PKA (20 U/ml) or cAMP (1 mM) shifted the activation midpoint (V1/2) of wild type HCN4 channels by ~10 mV to more depolarized voltages. No additional shift was produced when both cAMP and PKA were introduced together, suggesting that the two modulators share a common final pathway in channel activation. To characterize further the independent effects of each modulator, we used PKA-insensitive (HCN4-Cx4) and cAMP-insensitive (HCN4-R669Q) mutant HCN4 channels. In PKA-insensitive HCN4-Cx4 channels, cAMP significantly shifted the voltage-dependence to more positive potentials, similar to its effect in wildtype HCN4 channels. This result demonstrates that cAMP modulation of HCN4 does not require PKA phosphorylation of the distal C-terminus. In contrast, PKA had no effect on the voltage-dependence of activation in the cAMP-insensitive HCN4-R669Q channels. Taken together, the data are consistent with a model in which direct binding of cAMP and PKA phosphorylation share a final common gating mechanism, however PKA modulation of HCN4 requires an unmodified cAMP binding domain.

33) **β adrenergic regulation of If and heart rate in sinoatrial node myocytes is dependent upon AKAP tethered PKA.**

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The cardiac funny current, If, is produced by hyperpolarization-activated cyclic nucleotide sensitive (HCN) channels in sinoatrial myocytes, and is believed to contribute to β-adrenergic regulation of heart rate. Expression of HCN channels has been shown to be altered in the failing heart, and the resulting changes in If have been suggested to contribute to arrhythmias associated with heart failure. It is well established that β-adrenergic stimulation shifts the voltage dependence of If to more positive potentials. Our previous studies have shown that this modulation depends on the cAMP-activated protein kinase (protein kinase A, PKA). In many systems, PKA is anchored near its targets by A-Kinase anchoring proteins (AKAPs). Here, we have investigated the role of AKAPs, specifically AKAP150, in the modulation of If. We found that the ability of β-adrenergic regulation of If was significantly reduced by Ht31, an inhibitory peptide that disrupts AKAP-PKA complexes. Furthermore, AKAP150 was shown to immunoprecipitate with HCN4 channels from mouse sinoatrial node homogenates. To specifically investigate the role of AKAP150 in β-adrenergic regulation of If,, we used knock-in mice bearing a mutation in AKAP150 that prevents it from binding PKA adrenergic signaling to If wasβ(AKAP150ΔPKA). We found that PKA mice compared toΔsignificantly reduced in myocytes from AKAP150 wildtype myocytes. To determine whether AKAP150 plays a role in heart rate regulation, we recorded surface EKGs from anesthetized mice. Remarkably, adrenergicβheart rate in AKAP150ΔPKA mice was insensitive to stimulation, whereas heart rate in wildtype mice showed robust positive

chronotropy. To determine if the lack of heart rate response was due to a sinoatrial node defect, we measured firing rate from individual sinoatrial myocytes, and again found the response to β-adrenergic stimulation was significantly diminished when compared to wildtype. Taken together, these data provide strong evidence for a role for AKAP150-tethered PKA in sympathetic regulation of heart rate and If in mice.

34) **Kv2.1 cell surface clusters are insertion and retrieval platforms for ion channel trafficking at the plasma membrane.**

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The Kv2.1 delayed rectifier K+ channel regulates electrical activity in nerve and muscle and has been postulated to play a non-conducting role in SNARE-mediated protein fusion in neuroendocrine cells. Kv2.1 is unusual among voltage-gated K+ channels in that it localizes to micron-sized clusters on the cell surface of neurons, atrial myocytes and transfected HEK cells. Within these clusters Kv2.1 is non-conducting. Here we examined the hypothesis that these surface structures are specialized platforms involved in the trafficking of membrane proteins to and from the cell surface. TIRF-based studies indicated that GFP-Kv2.1 containing vesicles directly tether to and deliver cargo in a discrete fashion to the Kv2.1 surface clusters in both transfected HEK and cultured hippocampal neurons. Qdot-based single molecule experiments indicated that the delivery and surface retrieval of Kv2.1 occurs at the perimeter of the surface clusters. Overall, 85±8.4% of newly synthesized channels in HEK cells and 84.9±10.4% in hippocampal neurons were inserted within 0.5 microns of the cluster perimeter even though the Kv2.1 clusters represent only 21.4 ±3.8% of the basal cell surface. When 132 continuously recycling Kv2.1 channels in HEK cells were examined, 96.2% were also inserted at the cluster perimeter. Unlike Kv2.1, the Kv1.4 K+ channel has a homogeneous cell surface expression in transfected cells. Demonstrating that the Kv2.1 clusters represent cell surface platforms for more than just Kv2.1 insertion and retrieval, the non-clustering Kv1.4 K+ channel also inserted into the HEK cell plasma membrane at the Kv2.1 cluster perimeter. Kv1.4 endocytosis also occurred at this region. Together, these results indicate that a non-conducting function of Kv2.1 is to form specialized cell surface microdomains that are involved in membrane protein trafficking. This study is the first to identify stable cell surface sites for ion channel delivery and retrieval in mammalian cells.

EXPERTISE: Single particle tracking, TIRF and confocal microscopy, neuronal culture

35) **A novel fluorescent protein and biotin tagged Nav1.6 channel allows analysis of voltage-gated sodium channel dynamics in hippocampal neurons.**

EJ Akin, AV Weigel, D Krapf, MM Tamkun. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO and Department of Electrical and Computer Engineering, Colorado State University, Fort Collins, CO.

Voltage-gated sodium channels (Nav) are essential for most neuronal excitability and areconcentrated at the axon initial segment (AIS) where they are thought to be responsible foraction potential initiation. While much is understood about channel structure and function, thereis limited information on how cells process sodium channel proteins in real time. Since the large sodium channelcDNAs are difficult to manipulate, the most elegant trafficking work to date has utilized chimericproteins containing the sodium channel ankyrin-binding motif fused to other membrane proteins.While these approaches address aspects of sodium channel localization, they cannot addresssodium

channel function and there is always the question of whether these chimeras faithfullyreproduce wild-type sodium channel behavior. Appropriately tagged full-length and functionalsodium channels are required if sodium channel cell biology is to advance. In the present study,Nav1.6 was tagged with GFP and an extracellular biotinacceptor domain (BAD) in order to allow for visualization and single particle tracking offunctional sodium channels on the surface of living cells. This Nav1.6 construct demonstratedwild-type Nav1.6 activity when expressed in hippocampal neurons. Confocal microscopyindicated the tagged channel trafficked efficiently to the cell surface and had a denseaccumulation at the AIS. Alexafluor 594-conjugated streptavidin binding indicated the surfacedensity of channels at the AIS was approximately 60 times greater than on the soma. Thisdensity compares with that for the endogenous Nav1.6 channel. Fluorescence recovery afterphotobleaching (FRAP) and single particle tracking showed that channels at the AIS hadrecovery time constants of greater than 2 hours. In summary, our study demonstrates the creation of a functional sodium channelconstruct with fluorescent protein and extracellular biotin reporters that has both wild-typetrafficking and biophysical properties. This construct will be utilized to examine channelturnover, trafficking, diffusion and location-dependent function in neuronal cells.

36) **An AKAP79/150:L-Type calcium channel interaction is required for CaN-NFAT nuclear signaling.**

JG Murphy, ML Dell'Acqua. From the University of Colorado Denver, Anschutz Medical Campus, Aurora, CO.

In neurons, L-type voltage gated Ca2+ channels (LTCC) play an important role in the induction of gene expression-dependent long-lasting forms of synaptic plasticity. LTCCs couple electrical excitation, through diverse intracellular signaling pathways, to transcription factor activation (e.g. cAMP response element binding protein [CREB] and nuclear factor of activated T-cells [NFAT]), ultimately resulting in gene expression changes. The A-kinase anchoring protein 79/150 (AKAP79/150) is localized within the postsynaptic density of excitatory neurons of the hippocampal formation through interactions with plasma membrane bound phospholipids, the actin cytoskeleton, and MAGUK adapter proteins (e.g. PSD-95 & SAP97). AKAP79/150 anchors the regulatory subunit (RII) of PKA and the catalytic subunit of the phosphatase calcineurin (CaN). In this configuration, PKA and CaN form an opposed signaling domain that modifies the phosphorylation state of local substrates. Interestingly, AKAP79/150 interacts directly with the LTCC subtype Cav1.2 through a modified leucine zipper motif (mLZ) at its C-terminus and a complementary site on the cytosolic C-terminal tail of the pore forming subunit of Cav1.2. Our lab initially described that the structural anatomy of this AKAP79/150-Cav1.2 complex coordinates PKA and CaN within a Ca2+-microdomain at the mouth of Cav1.2. Within this microdomain PKA and CaN work in opposition to regulate LTCC phosphorylation and activity. However, because this work relied on whole-cell current recordings at the soma, it remains unclear how AKAP79/150 anchored PKA and CaN regulate local Ca2+ elevations in spines and dendrites. To address this question, we employ fluorescence imaging of calcium within individual dendritic spines using a genetically encoded Ca2+ indicator. Here, we provide evidence of a regulatory role for AKAP79/150 in isoproterenol enhanced Ca2+ influx in dendritic spines. Additionally, we show that CaN anchoring to AKAP79/150, in complex with the LTCC, is required for NFAT dephosphorylation, transport into the nucleus, and NFAT-dependent transcription. We suggest AKAP79/150-dependent modulation of LTCC activity and local activation of NFAT in dendrites influences NFAT retrograde translocation to the nucleus and subsequent NFAT dependent transcriptional regulation.

The AKAP79/150-LTCC interaction may couple local postsynaptic stimulation to changes in gene expression that underlies long lasting forms of synaptic plasticity.
EXPERTISE: dissociated hippocampal culture, fluorescence microscopy, Ca2+ imaging

37) **Phosphodiesterases form a cAMP "shield" to regulate the funny current, If, in mouse sinoatrial myocytes**

JR St. Claire, Z Liao, E Larson, C Proenza. From the Department of Physiology & Biophysics, University of Colorado Anschutz Medical Campus, Aurora, CO.

Intrinsic sinus node function generates the spontaneous electrical signal that causes the heart to contract beat after beat. This spontaneous activity is increased upon sympathetic nerve stimulation during the fight-or-flight response to increase heart rate. Cardiac automaticity is maintained by the coordination of many molecular mechanisms constituting the pacemaking complex; one of these being the hyperpolarization-activated HCN channel, which underlies the cardiac funny current (If). If is regulated by both basal and β adrenergic receptor-mediated cAMP signaling within sinoatrial myocytes; and we have recently shown that sympathetic activation of If requires activity of the cAMP-dependent protein kinase (Protein Kinase A; PKA). In many systems, phosphodiesterases (PDEs) are important for precise spatial and temporal regulation of cAMP within specialized signaling compartments. In this study, we have investigated the role of PDEs in regulation of If by recording hyperpolarization-activated currents from isolated mouse sinoatrial myocytes in the presence and absence of β adrenergic stimulation and PDE inhibition. We found that PDE inhibition potentiated If under both basal and stimulated conditions, and this potentiation was present even upon inactivation of PKA. These results indicate the presence of a PDE "shield" surrounding HCN channels in the sinoatrial node, suggesting that PDE activity within sinoatrial myocytes could contribute to changes in heart rate under normal and pathological conditions.

Neuroendocrine

38) **Elevated tph2 mRNA expression in an amygdala priming model of chronic anxiety.**

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The basolateral amygdala (BL), a key source of afferents to the central amygdala, densely expresses receptors for corticotropin releasing factor (CRF), and regulates anxiety-like behaviors. Previous studies have demonstrated that repeated, daily activation of CRF receptors in the BL ("BL priming") of male rats results in development of a chronic anxiety-like state. We hypothesized that BL priming alters neural output from the amygdaloid complex, and ultimately changes the expression of tph2, the gene encoding the rate-limiting enzyme for serotonin synthesis, specifically in those subdivisions of the serotonergic dorsal raphe nucleus (DR) that have been linked to anxiety-like behaviors. To test this hypothesis, we implanted 16 adult, male rats with bilateral guide cannulae targeting the BL to prime them either with daily injections of vehicle (1% bovine serum albumin in 0.9% sterile saline, n = 8) or urocortin 1 (UCN1, 6 fmoles/100 nl vehicle per side, n = 8), a CRF receptor agonist, for 5 consecutive days. The rats' behavior was assessed in the social interaction (SI) test one day before, and 2 days after the intra-BL priming paradigm. A third group (n = 7) served as undisturbed home cage controls. All rats were rapidly decapitated 3 days after the last intra-BL

injection to analyze tph2 and slc6a4 (gene encoding the serotonin transporter, SERT) mRNA expression in the DR using in situ hybridization histochemistry. UCN1 priming markedly reduced SI time compared to vehicle and home cage controls, elevated tph2 mRNA expression in the caudal DR, ventral part (cDRV) and in the ventrolateral DR/ventrolateral periaqueductal gray (DRVL/VLPAG), but did not alter slc6a4 mRNA expression. In addition, tph2 mRNA expression in the DRVL/VLPAG was strongly correlated with reduced SI time. The DRVL/VLPAG is well-positioned to control panic-like behavior, and the correlation of tph2 expression and behavior suggests a direct role for the DRVL/VLPAG in the control of anxiety states. Hence, it is likely that an elevation of tph2 mRNA expression in the DRVL/VLPAG is crucial for the development of a chronic anxiety-state, including panic-like behavior.
EXPERTISE: in situ hybridization, behavioral testing, immunocytochemistry, cRNA design, neuroanatomy

39) **Deciphering the role of histone deacetylases in GnRH neuronal development.**
S Salian-Mehta[1], M Xu[1], Horn T[2], Mckinsey T[2], ME Wierman[1]. From the [1]Department of Medicine, Physiology and Biophysics, University of Colorado Denver, [2]Research Service Veterans Affairs Medical Center, Denver, and Division of Cardiology, University of Colorado Denver, Aurora, CO.
Epigenetic modulation of GnRH neuronal function across development is not well understood. DNA microarrays examined differential gene expression in migratory NLT neuronal cells with low levels of GnRH with post-migratory GT1-7 cells which have high GnRH expression. Pathway analysis revealed that the NLT cells expressed lower transcript levels of most histone deacetylases (HDACs) and DNA methyl transferases as compared to GT1-7 cells. HDACs are a family of deacetylases initially described as global transcriptional repressors, but with subfamily members having cell specific roles. Class I HDACs were increased in GT1-7 neuronal cells (HDAC1: 3376 vs. 1195, HDAC2: 3485 vs.1189). HDAC activity was assessed using acetylated class specific HDAC substrates that once deacetylated by the endogenous HDAC become susceptible to cleavage by trypsin and release a fluorophore. Consistent with alterations in gene expression, Class I HDAC activity in GT1-7 cells was increased (1.7 fold $p<0.05$). Overall Class IIa HDACs (4/5/7) transcripts and activity levels were similar between the neuronal cells, whereas Class IIa HDAC9 expression was increased 6.7-fold in GT1-7 cells (119 vs.17). HDAC6 (Class IIb) which in other systems plays a role in cell movement by deacetylation of tubulin was 3.5-fold higher in the GT1-7cells (893 vs249) compared to the NLT neurons. Confirming a functional significance, HDAC6 enzymatic activity was increased by 5.7- fold. HDAC inhibitors, TSA (blocks Class I/II), Tubastatin (blocks HDAC 6), MGCD0103 (blocks Class I), and DPAH (blocks Class II) had no effect on endogenous GnRH gene expression as assessed by RT-PCR. In migration assays, inhibitors of HDAC blocked migration in NLT cells. Incubation with HDAC inhibitors Tubastatin and DAPH in NLT and GT1-7 cells led to increased caspase 3 cleavage. HDAC9 is known to putatively influence MEF2 target genes by interacting with MEF2; hence we are currently investigating the effects of re-expression of hHDAC9 and MEF2-LUC in NLT and GT1-7 neuronal cells and studying their functional effects. Data will provide novel insights into the effects of HDACs during neuronal development.
EXPERTISE: IHC, ISH, PCR, tissue culture

40) **Gonadotrophin-releasing hormone system during pubertal transition of fibroblast growth facotr 8-deficient mouse.**

W Zhang, J Rochester, S Kavanaugh, P-S Tsai. From the Department of Integrative Physiology, University of Colorado at Boulder, Boulder, CO.

Gonadotropin-releasing hormone (GnRH) is critical for the onset and maintenance of reproduction in vertebrates. Puberty, a significant transition in life involving reproductive onset, is triggered by increasing levels of GnRH synthesis and secretion. The development of GnRH neurons is highly dependent on fibroblast growth factor (Fgf) signaling. In transgenic mice deficient in Fgf8 signaling (Fgf8 hypomorphs), the genesis of GnRH neurons is disrupted, leading to a 50% decrease in the number of postnatal GnRH neurons. However, the initiation of pubertal onset appears unaffected in these mice, suggesting the presence of compensatory mechanisms. In this study, we sought to understand the nature of these compensatory mechanisms by examining GnRH neuron number and hypothalamic GnRH content in Fgf8 hypomorphs and wildtype (WT) mice within ages encompassing pubertal transition (postnatal day (PN) 10, 20, 25, 30, 35, to 40). Our results showed that GnRH neuron numbers were significantly reduced in Fgf8 hypomorphs compared to WT in all age groups examined. In parallel, significant decreases in hypothalamic GnRH content in Fgf8 hypomorphs were observed in all age groups except PN35. Interestingly, during the pubertal transition at PN35, the GnRH content of Fgf8 hypomorphs increased to a level no longer different from that seen in WT mice. These data indicate that, during puberty, the GnRH system compensates for the loss of GnRH neurons by increasing its overall GnRH production. This speaks to the extraordinary ability of an organism's pubertal drive to overcome pre-existing deficiencies in order to ensure its reproductive onset. (Supported by NIH 042634 to PST)
EXPERTISE: Immunocytochemistry and PCR.

41) **Localization of the expression of gonadotropin-releasing hormone like-molecule in a gastropod mollusk aplysia californica.**

L Jung, S Kavanaugh, P-S Tsai. From the Department of Integrative Physiology, University of Colorado, Boulder CO

Gonadotropin- releasing hormone (GnRH) is a key neuropeptide for regulating reproduction in vertebrates. The recent discoveries of GnRH-like molecules in non-chordate animals suggested GnRH may have arisen in an ancestral bilaterian that gave rise to both protostomes and deuterostomes. Our laboratory has previously isolated a full-length cDNA of a GnRH-like molecule, named ap-GnRH, from a gastropod mollusk, Aplysia californica. Immunocytochemistry revealed the presence of neurons positive for ap-GnRH in only 2 central ganglia, the pedal and cerebral ganglia, of A. californica, but a specific radioimmunoassay revealed that all 5 central ganglia contained immunodetectable ap-GnRH, leading to some confusion regarding the source of this peptide. The goal of the present study is to localize ap-GnRH transcript in central and peripheral tissues of A. californica by in situ hybridization (ISH). Using a 604 nt-cRNA probe, we detected the strong presence of ap-GnRH transcript in neurons of the pedal ganglia. In addition, many positive neurons were localized in the cerebral ganglia, followed by 1-4 neurons in the abdominal ganglia. No staining was observed in the remaining central ganglia (buccal and pleural ganglia) or in the peripheral tissues (the ovotestis and atrial gland). These data are largely consistent with our previous immunocytochemical data and support the presence of both ap-GnRH peptide and transcript in the pedal and cerebral ganglia. However, the presence of ap-GnRH mRNA in the abdominal ganglia suggests transcriptional activity also occurs in these ganglia. As such, our inability to detect abdominal ap-GnRH immunoreactive neurons may reflect either alternative processing or exceptionally fast turnover of the peptide in these

ganglia. Overall, our data suggest that ap-GnRH is produced by multiple ganglia and support the notion that ap-GnRH may assume functions beyond reproduction.
(Supported by NSF IOS 0743818 to PST)
EXPERTISE: Immunocytochemistry and In situ hybridization

42) **Opposite-sex cohabitation restores the aging GnRH system and promotes the morphological maturation of GnRH neurons in transgenic animals.**

JR Rochester, P-S Tsai. From the Department of Integrative Phyisology, University of Colorado, Boulder, CO.

Gonadotropin-releasing hormone (GnRH) is a neuropeptide that drives reproduction in all vertebrates. Fibroblast growth factors (FGFs) are crucial for the development of GnRH-secreting neurons. Further, preliminary data have shown that FGFs are also important in the postnatal maintenance of GnRH neurons. We previously generated transgenic dnFGFR mice, which have reduced FGF receptor function at the level of the GnRH neuron. While wildtype (WT) mice maintain the same number of GnRH neurons throughout their lives, dnFGFR mice show an age-dependent decline of these neurons. Interestingly, we found that opposite-sex (OS) cohabitation restores GnRH neuron number and peptide concentration to WT amounts in male dnFGFR mice. In this study, we explored the effects of OS housing on GnRH neuron morphology as a marker of GnRH neuron functionality in dnFGFR male and female mice. Although WT mice do not lose GnRH neurons over time, these neurons undergo extensive morphological remodeling during the pubertal transition. During puberty, the number of complex neurons decreases while the number of unipolar neurons increases, likely indicating a functional maturation of the GnRH system. We examined the morphological distribution of GnRH neurons in dnFGFR mice at PN35, and found that these mice exhibit a more “juvenile” distribution than WT, with more complex and fewer unipolar neurons. This altered distribution persisted in PN100 males. However, PN100 males housed with females since puberty showed normal distribution of GnRH neuron types. dnFGFR PN100 females seemed to show opposite effects, with increased complex and decreased unipolar neurons in dnFGFR OS females but a more "mature" distribution of neruons in SS females. Our data show, for the first time, that FGF signaling is crucial for the normal morphological changes in maturing GnRH neurons. Further, environmental cues (i.e. opposite-sex housing) can override defects in the FGF system to drive GnRH system maintenance and maturation in males. These data indicate that the GnRH system is highly plastic and responsive to the external environment throughout adulthood, and that lifestyle choices may promote fertility in aging individuals.
EXPERTISE: Immunocytochemistry, PCR, real time qPCR, cell culture, surgery

43) **Short term exposure of a high dose GnRH agonist and its effects on gonadotropin secretion in the anterior pituitary of adult male rats**.

BS Edwards[1], CS Asa[2], DC Skinner[1]. From the [1]Neuroscience Program and Department of Zoology and Physiology, University of Wyoming, Laramie, WY; [2]Research Department, Saint Louis Zoo, Saint Louis, MO

GnRH agonists are used in the treatment of steroid-dependent gonadal diseases in men and as a contraceptive for other mammals. Agonists work through desensitizing gonadotropes in the anterior pituitary, which suppresses the synthesis and/or release of FSH and LH. The decrease in FSH and LH has a direct effect on testosterone (T) production. There is evidence that FSH and T significantly ($P<0.01$) increase within 4 weeks following low dose (1.1mg) deslorelin treatment. Here, we investigated the effect of a high deslorelin dose on the reproductive axis of male rats. Male Sprague Dawley rats (185.2 ± 0.4 days) were treated with 14.1 mg deslorelin implants for a period

of 44.4±0.4 days and separated into 2 post-treatment groups: 1) sacrificed immediately (n=7), 2) sacrificed after 4 weeks recovery (n=6). Another group (n=6) were treated with 14.1mg deslorelin plus a 15mg T implant. Five sham implanted rats were used as controls. Using immunocytochemical techniques, pituitary sections were labeled for DAPI, FSHβ and LHβ. Deslorelin treatment suppressed ($p<0.05$) the percentage of FSHβ mono- and bi-hormonal cells immediately after treatment, resulting in a significant increase in the percentage of mono-hormonal LHβ cells. The deslorelin recovery groups showed no significant difference in both mono-hormonal and bi-hormonal gonadotropes when compared to controls. Plasma levels of T, FSH, and LH were determined by RIA or ELISA. Both FSHβ and LHβ mono-/bi-hormonal immunoreactive cells were significantly reduced following treatment, but recovery occurred within 4 weeks. T and FSH were significantly ($p<0.05$) reduced compared to the 4 week recovery and control groups. Interestingly, T rescued the FSH plasma concentration although FSHβ immunoreactivity was reduced compared to controls, suggesting that remaining gonadotropes produce more FSH in presence of T. These data show that high dose deslorelin for 6 weeks suppresses the reproductive axis but this effect is reversed within 4 weeks. We are currently investigating the effects of duration of exposure to high dose deslorelin.
EXPERTISE: Immunocytochemistry, ELISA, RIA

44) **Gonadotropin-releasing hormone receptor in Aplysia californica.**

SI Kavanaugh, P-S Tsai. From the Department of Integrative Physiology and Center for Neuroscience, University of Colorado, Boulder, CO.

Gonadotropin-releasing hormone (GnRH) is a universal activator of the vertebrate reproduction. However, a recently elucidated GnRH-like molecule in the mollusk, Aplysia californica, does not appear to have a reproductive role. To better understand the function of Aplysia GnRH and its target tissues, we attempted to identify candidates for the GnRH receptor (GnRHR) in A. californica. Initially, we performed in silico analysis of expressed sequence tags and whole genome shotgun sequences to search for A. californica sequences similar to other known Trochozoa GnRHRs or GnRHR-like receptors. Four partial sequences with high identity to GnRHRs were found. Attempts were made to expand these sequences using a trace assembler (http://genotrace.niob.knaw.nl/). The only significant match was found between an A. californica sequence and the N-terminus of the octopus GnRHR. Using this partial sequence, we designed degenerate primers to amplify a larger segment of the target gene and subsequently isolated its 3' end using the 3' rapid amplification of cDNA ends. The identified receptor transcript maintains the conserved structural features and motifs of other known type II GnRH receptors and has 60% identity with the octopus GnRHR. Interestingly, intracellular loop 3 of the A. californica GnRHR-like receptor is approximately 32 amino acids (aa) longer than that of the octopus GnRHR. The C-terminal tail is approximately 44 aa long, which is 35 aa shorter than the octopus GnRHR. Expression of the receptor transcript is confined to the central nervous system. Phylogenetic analysis places the A. californica GnRHR-like receptor with the octopus GnRHR in the GnRH / corazonin receptor families. To date, the only GnRHR candidate identified in A. californica is the one described here. Future plans are to characterize its expression using in situ hybridization and conduct functional studies to establish its identity as a bona fide GnRHR. Supported by NSF IOS-0743818 to P-S. Tsai
EXPERTISE: Immunocytochemistry, in situ hybridization, Radioimmunoassay, PCR

45) **Sex differences in the central control of HPA axis responses is dependent on stressor modality**

JA Babb, CV Masini, HEW Day, S Campeau. From the Department of Psychology and Neuroscience, University of Colorado Boulder, Boulder, CO.

Women are more susceptible to developing certain mental illnesses than men, such as depression and several anxiety disorders. Stress is a causative and/or exacerbating factor for many diseases, and studies in rats have shown that females have higher neuroendocrine responses than males to various psychological stressors, such as restraint and loud noise. Previous research in our laboratory has characterized the hypothalamic-pituitary-adrenocortical (HPA) axis response to acute noise stress in males. Although previous literature shows robust sex differences in neuroendocrine responses to this stressor, we have been unable to replicate this effect across a wide range of noise intensities. The following experiments were performed to determine whether different stressor modalities (noise or restraint stress) affect the magnitude of sex differences in HPA axis activation, at both the hormonal and central levels. In the first experiment, young adult male and female rats in either diestrus, proestrus or estrus were exposed to restraint for 30 min. In the second experiment, young adult male and female rats in proestrus were exposed to 30 min of 95 dBA noise or home cage control conditions. Following each stressor, blood plasma and 10 µm brain slices were analyzed for hormone concentration and c-fos mRNA expression, respectively. Both noise and restraint significantly increased adrenocorticotropic hormone (ACTH) and corticosterone (CORT). Following restraint, females had significantly higher ACTH, CORT, and c-fos mRNA expression in the paraventricular nucleus of the hypothalamus (PVN), anteroventral region of the bed nucles of the stria terminalis (BSTav) and the medial preoptic area (MPOA) than males. There was no effect of estrous cycle stage for any measure. In contrast to restraint stress, there was no main effect of sex on ACTH, CORT, or PVN c-fos mRNA expression following loud noise. Analysis of the effect of sex on c-fos mRNA expression in other brain regions such as the BSTav and MPOA following noise stress is in progress. These data suggest that male and female rats may respond differently to acute psychological stressors of different modalities at both the neuroendocrine and central levels.

EXPERTISE: Dual Fluorescent in situ hybridization (FISH), female rat techniques including estrous cycle monitoring

46) **Anti-apoptotic role of ceramide synthase 6 (CerS6) in gonadotrope pituitary tumors**

K Kiseljak-Vassiliades[1,4], M Xu[1,4], AJ Knox[1], KA Michaelis[1], BK Kleinschmidt-DeMasters[2], KO Lillehei[3], ME Wierman[1,2]. From the Departments of [1]Medicine, [2]Pathology, and [3]Neurosurgery, University of Colorado Denver, Aurora, CO; [4]Research service, Division of Endocrinology, Veterans Affairs Medical Center, Denver, CO.

Pituitary tumors are associated with significant morbidity by local invasion leading to visual loss and pituitary insufficiency. Molecular mechanisms underlying pituitary tumorigenesis are poorly understood. We performed gene expression DNA microarrays on 14 gonadotrope tumors and 9 normals to identify genes involved in tumorigenesis or progression. CerS6 (ceramide synthase 6), was identified as a novel candidate with significant overexpression (6.6 fold) in gonadotrope tumors compared to normals. Comparing nonaggressive (NON) and aggressive (AGG) gonadotrope tumors, we found CerS6 to be significantly higher in AGG tumors (1.73 fold), implicating CerS6 as a potential prognostic marker. CerS6 is one of six ceramide synthase enzymes responsible for ceramide synthesis and also modulating events such as apoptosis and differentiation. CerS6 mRNA levels are elevated in breast, and head and neck cancer,

while suppressed in lung cancer. Our hypothesis is that CerS6 overexpression has pro-survival role in pituitary gonadotrope tumors. To assess its functional role in pituitary cells, we transiently overexpressed CerS6 in the immortalized mouse gonadotrope cell line (αT3). CerS6 overexpression protected cells from 48-hr serum withdrawal induced apoptosis as assessed by caspase 3 cleavage (2.2-fold). CerS6 silencing, conversely, increased apoptosis at 72h as assessed by caspase 3 cleavage (1.9-fold). To examine potential tumorigenicity of CerS6, cells stably overexpressing CerS6 were plated on soft agar. Following 22 days, CerS6-stable cells formed increased number of colonies compared to vector controls (26.3+/- 2.9, 14.3+/-2.3, respectively $P<0.03$), a 1.9- fold increase in colony formation. In summary CerS6 is significantly higher in gonadotrope tumors compared to normals. Our data suggest that overexpression of CerS6 has anti-apoptotic and tumorigenic effect in gonadotrope cells, positioning CerS6 as a promising candidate in pituitary tumorigenesis, and potential prognostic and therapeutic target.
Funded by VA Merit Review to MEW, UCD Cancer Center and Genomics Core

47) **The nonpathogenic, saprophytic bacterium, Mycobacterium vaccae, selectively activates a subset of serotonergic neurons in the dorsal raphe nucleus in association with hypothermia in mice**

PHW Siebler, MW Hale, JL Lukkes, CA Lowry. From the Department of Integrative Physiology and Center for Neuroscience, University of Colorado Boulder, Boulder, CO.
Peripheral immune activation can have profound physiologic and behavioral effects including induction of fever and sickness behavior. One mechanism through which immune activation or immunomodulation may affect physiology and behavior is via actions on brainstem neuromodulatory systems, such as serotonergic systems. In this study we demonstrated that peripheral immune activation with antigens derived from the nonpathogenic, saprophytic bacterium, Mycobacterium vaccae, activated a specific subset of serotonergic neurons in the interfascicular part of the dorsal raphe nucleus (DRI) of mice, as measured by quantification of c-Fos expression 13.5 h following administration of heat-killed, intact M. vaccae. Furthermore, it was shown that administration of M. vaccae resulted in a hypothermic response 10 – 10.5 h following treatment which may suggest the activation of a thermoregulatory cooling mechanism. Serotonergic neurons within the DRI are known to project to forebrain structures involved in the control of thermoregulation and the control of cognitive function and mood. These data are in agreement with previous findings in which M. vaccae administration resulted in reductions of immobility in the forced swim test, which indicates an antidepressant-like behavioral response.
EXPERTISE: behavior testing, perfusions, tissue sectioning, immunohistochemistry, microscopy

Sensory and Motor Systems

48) **Cross-modal re-organization in adults with mild hearing loss.**

J Campbell, L Durkee, A Sharma. From the Speech, Language and Hearing Sciences Department, University of Colorado, Boulder, CO.
Background: Approximately 36 million adults in the U.S. suffer some form of hearing loss (NIDCD, 2010). However, only one out of five people with hearing loss who may benefit from hearing aids actually wears these devices (NIDCD, 2010). Most patients with hearing loss wear hearing aids only when their hearing loss has progressed to a severe degree, resulting in a significant hearing handicap and dissatisfaction with the use of amplification. Aim: Research in adults with severe-profound hearing loss and deafness

has shown that areas of auditory cortex may be recruited by the visual modality for visual processing. This cross modal re-organization leads to a competition of available resources if auditory input is to be re-introduced via amplification devices. Though cortical re-organization has been studied extensively in deaf adults, there is a striking absence of research investigating the degree of hearing loss at which cortical re-organization may be instigated. Such re-organization may then impact the success of the later fitting of hearing aid amplification, preventing beneficial use of these devices. The goal of this study was to examine re-organization via EEG in adults with milder degrees of hearing loss and to correlate measures of re-organization with outcome. Method: Normal-hearing adults and adults with a mild hearing loss (in the low-mid frequency range) wore a 128-channel electrode net while observing a visual pattern. From this EEG recording, topographic maps of mean voltage values were generated across the scalp, showing the extent of visual cortical activation in response to the pattern. The same subjects also listened to a nonsense syllable in order to determine functional integrity of the auditory cortical pathways. Speech perception was measured using a sentence list presented in varying levels of background noise. Results: Amplitude and topography for the cortical potential components were significantly different for adults with hearing loss. Behavioral outcomes reflected by speech perception in noise scores were significantly worse for the patients with hearing loss. Results show significant changes in cortical potentials for patients with hearing loss suggestive of higher-order cortical re-organization.
EXPERTISE: EEG testing with EGI and Neuroscan systems, EEG analysis in Matlab, ICA, behavioral testing in hearing loss, pediatric EEG testing, design of auditory and visual stimuli

49) **Mechanisms of cholinergic modulation of midbrain motor output: An optogenetic approach.**

EA Stubblefield, Gidon Felsen. From the Depts. of Physiology and Biophysics, University of Colorado Denver, Aurora, CO

Neural circuitry has evolved to generate well-controlled motor function with exquisite precision. Mammals requires acute sensory input to neural networks that must fire in rapid order to drive coordinated motor output. This rapid order of neural firing is crucial for the sequential components of motor output: movement selection, preparation, and execution. Movements are controlled by several inter-related pathways. The intermediate layer of the superior colliculus (SC) controls movement initiation and selection across species (Wurtz & Goldberg; Felsen & Mainen). The SC receives cholinergic input from the pedunculopontine tegmental nucleus (PPT), but the function of this input is poorly understood. In primates, increased acetylcholine levels in the SC increase the speed in which movements are initiated, suggesting this major cholinergic projection may underlie the preparation of movements. Until now, examination of this cholinergic projection from the PPT and subsequent firing activity of midbrain SC premotor neurons was not feasible. Optogenetic tools now make it possible to manipulate specific neural projections with spatio-temporal precision. Utilizing these tools in conjunction with in vivo electrophysiology and behavioral studies, I test the hypothesis that selective activation of cholinergic input from the PPT to the intermediate gray layer of the SC increases the firing rate of principal SC premotor neurons (SGI neurons) that underlie movement preparation. To selectively excite only cholinergic PPT neurons, I inject transgenic ChAT-Cre mice with a viral construct containing floxed channelrhodopsin (ChR2) into the PPT. Once ChR2 expression is robust, an optic fiber is implanted into PPT axon terminals entering the SC, and recording tetrodes are then carefully positioned within recipient intermediate SC principal neurons. Thereafter, in

vivo recordings are conducted during behavioral, movement-based tasks, already demonstrated in rats (Felsen & Mainen,). My goal is to establish an optogenetic model for in vivo recordings in awake, behaving mice. Taken together, we will gain a better understanding of how this anatomically conserved cholinergic pathway leads to neuronal activity underlying movement preparation and execution.
EXPERTISE: I have extensive experience conducting whole-cell patch clamp recordings in acute slices as well as from cultured neurons. I also now conduct in vivo electrophysiology, mouse behavior, and mouse brain surgeries for implanting optic fibers and recording tetrodes within the midbrain/brainstem areas. I'm currently learning transcardial perfusions, histology, fluorescence microscopy, and I maintain a transgenic ChAT-Cre mouse colony.

50) **Sub-sensory mechanical noise input to ankle tendons improves movement detection.**

BL Tracy, AM Grossman, AA Amin, JL Uphoff, SK Anast, RJ Paxton. From the Department of Health and Exercise Science and College of Applied Human Sciences, Colorado State University, Fort Collins, CO.

Sub-sensory mechanical noise to the DF and PF tendons improved detection of ankle joint movement in both directions in the sagittal plane. Movement detection was slightly better for the DF than PF direction. Vibration (VIB) improved movement detection more for those with the worst movement detection. The data suggest that subliminal mechanical input to tendons can improve proprioception, perhaps by increasing the sensitivity of muscle spindles to changes in muscle length during joint movement. Young men (N=22, 21.0 ± 2.3 yrs) and women (N=20, 21.6 ± 2.4 yrs) with no history of serious ankle sprains underwent assessment of joint movement perception threshold (JMPT) in the dominant ankle. A motorized, instrumented platform slowly (0.25 deg/s) rotated one ankle joint from a starting position of 90 degrees. Standing and listening to noise via headphones, subjects pushed a stop button the moment they perceived ankle movement. The degrees of movement were recorded as JMPT. Two trials were performed in the direction of plantarflexion (PF) and dorsiflexion (DF), with (VIB) or without tendon vibration (NOVIB). VIB was applied to the Achilles and tibialis anterior tendons via small vibrating discs (tactors driven with white noise) embedded in an elastic ankle strap. VIB amplitude was delivered during the test trials at just below the subject-reported cutaneous sensory threshold. The order of VIB and movement direction was counterbalanced. Pooled across movement directions, JMPT was less for VIB than NOVIB (1.12 ± 0.17 vs.1.40 ± 0.21 deg, P=0.002). The effect of VIB was similar for DF (0.93 ± 0.09 vs. 1.14 ± 0.15 deg) and PF (1.31 ± 0.27 vs. 1.67 ± 0.28 deg). Pooled across VIB conditions, JMPT was less for the DF than PF direction (1.03 ± 0.11 vs. 1.49 ± 0.27 deg, P=0.02). JMPT was correlated between DF and PF direction (r=0.72, P<0.0001). The difference in JMPT between VIB and NOVIB was negatively correlated with JMPT for the DF (r=-0.82, P<0.0001) and PF (r=-0.32, P=0.04) directions.
Supported by NIH AG035147

51) **Characterization of axo-axonic synapses in the piriform cortex of musculus.**

X Wang, Qian-quan Sun. From the Department of Zoology and Physiology and the Neuroscience Program, University of Wyoming, Laramie, WY

Previous anatomical studies have established major glutamatergic and GABAergic neuronal subtypes within the PC circuits. However, the quantitative properties of axo-axonic inhibitory synapses (AAIS) mediated by chandelier cells across major cortical subdivisions of PC are unknown. Therefore, we examined the properties of AAIS across the entire PC. Our results show: 1) GAT1-positive varicosities whose

appearance resembles to chandelier cell cartridges are formed around the ankyrin-G positive initial segment of axons (AIS) of glutamatergic cells across layers II and III of PC. 2) Both the density of AAIS cartridges and the degree of GAT-1 innervations in every AAIS is significantly higher in the PC than motor cortex. 3) We found that GAD67, VGAT, and PV but not calbindin, are co-localized with the presynaptic varicosities, whereas gephyrine, NKCC1, and GABAA alpha 1 & 2 but not KCC2, are colocalized at the presumed postsynaptic sites of AAIS. 4) The AAIS cartridges are expressed in majority of excitatory neurons and are distributed more frequently in putative centrifugal vs. non-centrifugal cortical cells. 5) We described morphology of chandelier cells using PV-IR and single cell labeling techniques and identified bituffed fast-spiking interneuron as chandelier cells. We conclude that very small number of chandelier cell mediated abundant AAISs across the entire PC. Due to the critical location of AAIS in relation of action potential regulation in glutamatergic cell, our results highlight a critical role of AAIS in regulating information flow and olfactory-related oscillation within the PC in vivo.
EXPERTISE: Immunostaining

52) **Risk, variability, and decision-making in goal-directed movements**

MK O'Brien, AA Ahmed. From the Department of Integrative Physiology, University of Colorado Boulder, Boulder CO.

In this study, we examined the effects of risk and variability on movement planning during goal-directed arm-reaching and whole-body movements. We have designed a task that is analogous to approaching the edge of a cliff, where your proximity to the cliff edge involves a tradeoff between the reward afforded by the view and the penalty incurred by falling over the edge. Healthy young adult subjects make swift arm-reaching movements or use their center of pressure to move a cursor as close to the edge of a virtual cliff as possible without going over the edge. They receive points based on the cursor's final proximity to the cliff edge. We test four conditions: (1) "null," where there is no point reward nor penalty if the cursor enters the cliff region; (2) "noise," where the cursor dynamics include Gaussian random noise; (3) "cliff," where subjects receive a significant point penalty if the cursor enters the cliff region; and (4) "cliff+noise," where the cursor dynamics include noise and subjects receive a point penalty for entering the cliff region. We also determine each subject's endpoint variability as a function of movement distance. We then compare subjects' movement endpoints to endpoints predicted by a subject-specific model of optimal movement planning, which is based on principles of statistical decision theory. We observe that increasing variability and explicit risk does affect movement endpoints for both arm-reaching and whole-body movements in this goal-directed cliff task. Furthermore, our endpoint model is capable of approximating endpoints for arm-reaching (indicating that arm-reaching movements are optimal). In the whole-body task, subjects tended to move slightly closer to the cliff than the model predicted for most conditions, particularly in "cliff" and "cliff+noise" (indicating that whole-body movements are near-optimal). By comparing subjects' actual movement endpoints to their model-predicted optimal endpoints, we can quantify subjective risk valuation and determine if individuals are inappropriately risk-averse or risk-seeking.

53) **Movement adaptation under conditions of risk and instability**

MC Trent, AA Ahmed. From the Neuromechanics Lab, Department of Integrative Physiology, University of Colorado, Boulder, CO.

Not all movement errors are created equal. For example, compare a 5 cm error in foot placement when approaching the edge of a curb, to the same error approaching the edge of a cliff. The risk, or subjective value associated with the error, is different. One

would likely avoid the cliff edge more than the edge of the curb. In other words, movement adaptation would depend not only on the magnitude of the error but also on the penalty (subjective value) associated with the error. Interestingly, models of adaptation have traditionally assumed that adaptation is proportional to movement error . In recent years the notion of proportionality been challenged. However, the role of risk in movement adaptation has not been investigated. Here we quantified adaptation in a unique cliff-like virtual environment that was stable, but only within certain stability limits, to influence the risk associated with a given movement error. Subjects made planar 15cm reaching movements to a target directly ahead of them. Cursor, start and target locations were presented on a monitor at eye-level. Curl field gains varied from trial to trial and were drawn from a uniform distribution ranging from 0 to -40 Ns/m, biased to the left. The unstable environment simulated reaching along the edge of a virtual cliff (stability limits). Curl field dynamics were maintained, but rightward errors greater than 2.5cm were penalized. A line was displayed on the monitor at the cliff edge, but no explicit instructions were given regarding the boundary. Importantly, almost all movement errors in both stable and unstable conditions were less than 2.5 cm. Subjects were merely alerted to the presence of the cliff, but rarely encountered it. To adapt subjects must produce a rightwards force, towards the instability. In the unstable environment, over adaptation is penalized in that it can lead to errors beyond the edge. We hypothesized that errors closer to the edge would be penalized more heavily than errors of the same magnitude that occurred when the instability was not present. Counter-intuitively, this would lead to reduced adaptation to the stronger gains.
EXPERTISE: Robot reaching tasks

54) **Does actual metabolic cost decrease with motor learning?**

HJ Huang, R Kram, AA Ahmed. From the Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO.

It is often assumed that the central nervous system controls movements in a manner that minimizes energetic cost. While empirical evidence for actual metabolic minimization exists in locomotion, actual metabolic cost has yet to be measured during motor learning and/or arm reaching. Here, we measured metabolic power consumption using expired gas analysis, as subjects learned novel arm reaching dynamics. We hypothesized that 1) metabolic power would decrease with motor learning and 2) muscle activity and coactivation would parallel changes in metabolic power. Seated humans made horizontal planar reaching movements towards a target using a robotic arm. The novel dynamics involved compensating for a viscous curl force field that perturbed reaching movements. Metabolic power was measured continuously throughout the protocol. Subjects decreased movement error and learned the novel dynamics. By the end of learning, net metabolic power decreased by ~20% (~0.1 W/kg) from initial learning. Muscle activity and coactivation also decreased with motor learning. Interestingly, distinct and significant reductions in metabolic power occurred even after muscle activity and coactivation had stabilized and movement changes were small. These results provide the first evidence of actual metabolic reduction during motor learning and for a reaching task. Additionally, they demonstrate that movement and muscle activity are not as tightly coupled with metabolic cost as previously thought. Other mechanisms besides changes in movement and muscle activity may also underlie the reduction of metabolic cost during motor learning. Efficient neural processes such as neuronal signaling in the brain could contribute to the reduction in metabolic cost.
EXPERTISE: surface electromyography, force plate analyses, indirect calorimetry, motor learning, virtual environments, building mechatronic devices, computer simulations

55) **Timing-dependent suppression of mitral cell output by inter-glomerular lateral inhibition targeted on external tufted cells**

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Sensory input from the olfactory nerve arrives at glomeruli in the olfactory bulb (OB) with variable timing such that some glomeruli receive input tens to hundreds of milliseconds before others. Because the OB includes dense networks of GABAergic interneurons, we wondered whether glomeruli activated by an earlier input could laterally inhibit those that receive later input. To test this, we used patch clamp recordings in rat OB slices, examining the effect of stimulation of a "conditioning" glomerulus applied 20 ms before stimulation of a "target" glomerulus (200-1200 μm separation between glomeruli). This dual stimulation resulted in a 25±5% decrease in the evoked excitatory current in mitral cells (MCs) located at the target glomerulus (n=26, $p<0.001$). We performed microsurgical cuts to determine the cell-type(s) mediating this lateral inhibition and found that inhibition could still be observed in slices with a cut through the external plexiform layer (EPL; 18±6% decrease, n=13, p=0.01), but a cut through the glomerular layer abolished lateral inhibition (1±2% decrease, n=9, p=0.9), implicating glomerular layer cells rather than granule cells. To determine which cells are the targets of lateral inhibitory input, we made whole-cell recordings from external tufted (ET) cells and MCs while uncaging glutamate at a glomerulus 100 to 500 μm away with a UV laser in the presence of MNI-caged-glutamate. We observed inhibitory post-synaptic currents (IPSCs) in ET cells (469±165 pA*ms, n = 38 uncaging spots in 8 cells; $p<0.005$) but in MCs there was no IPSC observed within the first 100 ms after stimulation (-61±58 pA*ms, n=45 uncaging spots in 9 cells, p=0.84). The duration of ET cell inhibition elicited by electrical stimulation of a conditioning glomerulus (1/2 w = 31±5 ms) correlated well with the time-interval (20 ms) between conditioning and test stimulation that was most effective for suppression of MC excitation. Because ET cells have a prominent role in mediating feed-forward excitation between OSNs and MCs (De Saint Jan et. al., 2009), we propose that lateral inhibitory input targeted on ET cells may impact the OB's input-output relationship by preventing feed-forward excitation of MCs.

EXPERTISE: patch clamp, cloning, confocal imaging, biochemistry

56) **GABA-induced calcium transients in juxtaglomerular neurons of the mouse olfactory bulb**

PV Parsa, RD D'Souza, S Vijayaraghavan. From the Physiology and Biophysics Department, University of Colorado, School of Medicine, Aurora, CO

Periglomerular (PG) cells are the inhibitory interneurons of olfactory bulb glomeruli. While the existence of dendrodendritic synapses between PG cells has been demonstrated (Murphy et al. 2005), their functional role in olfactory information processing has not been well examined. We have demonstrated that activation of glomerular nicotinic acetylcholine receptors (nAChRs) leads to increased γ-Aminobutyric acid (GABA) release on to a majority of PG cells. Our results suggest that the PG-PG dendrodendritic synapse is common, and that cholinergic modulation of this synapse plays a key role in olfactory processing. It has been speculated that GABA depolarizes PG cells, but due to shunting of membrane conductance, their firing is inhibited (Smith and Jahr, 2002). However, such sub-threshold depolarizations could potentially lead to transmitter release. Using Ca2+ imaging in olfactory bulb slices, we show that upon focal glomerular application of 1 mM GABA, there is an increase in intracellular Ca2+ levels ([Ca]i) in juxtaglomerular (JG) neurons. This increase in [Ca]i was blocked by 20 μM gabazine suggesting a GABAA receptor-mediated depolarization of these cells. Ca2+ channel blockers (0.5 mM NiCl2, 50 uM Nimodopine) blocked these Ca2+ transients

significantly. We hypothesize that potential GABA-induced-GABA-release (GIGR) mechanisms can lead to global inhibition within the glomerulus via local excitation of PG cells. Amplification of GABA release would contribute to the modulation of glomerular output.Funded by NIH RO1 DC008855 (SV).

57) **Peripheral anatomy of a distinct class of rapidly adapting sinus hair follicle afferents**

CC Cassidy, CJ Woodbury. From the Graduate Neuroscience Program, Department of Zoology and Physiology, University of Wyoming, Laramie, WY.

A fundamental cornerstone of the field of cutaneous sensation is that sensory receptors are functionally specific and that morphologically distinct endings have discreet functional properties. Unfortunately, our knowledge of definitive structure/function relationships of cutaneous receptors is limited; most of our current understanding is either drawn from neonatal animal work (Woodbury & Koerber, JCN 505:547, 2007) or based largely on conjecture. To determine the functional properties of anatomically defined terminals in mature animals, we developed a novel in vivo mouse preparation with which the peripheral anatomy of physiologically identified neurons could be studied. Intrasomal recordings were obtained from trigeminal ganglion neurons in decerebrate, paralyzed, and artificially ventilated mice. Functionally characterized cells were iontophoretically filled with Neurobiotin, and after sufficient time for diffusion, skin containing the receptive fields was dissected out and processed and the terminals subsequently examined. To date, we have recovered terminals from 65 physiologically characterized skin sensory neurons in adult mice. Here we report novel findings on a distinct set of afferents innervating vibrissal sinus hair follicles. These fibers enter near the base of the follicle, course unbranched along the hair, and finally terminate as simple club-like endings at the ringwulst. This unique set of afferents was only recently described (Ebara et al., JCN 449:103, 2002) and predicted to exhibit slowly adapting response properties. Our studies, however, prove that they are actually exquisitely sensitive, rapidly adapting fibers. Our data reveal that this new in vivo mouse preparation is a powerful tool that will help determine the functional characteristics of cutaneous receptors and ultimately expand our understanding of sensory transduction in the skin. Supported by NS44094.