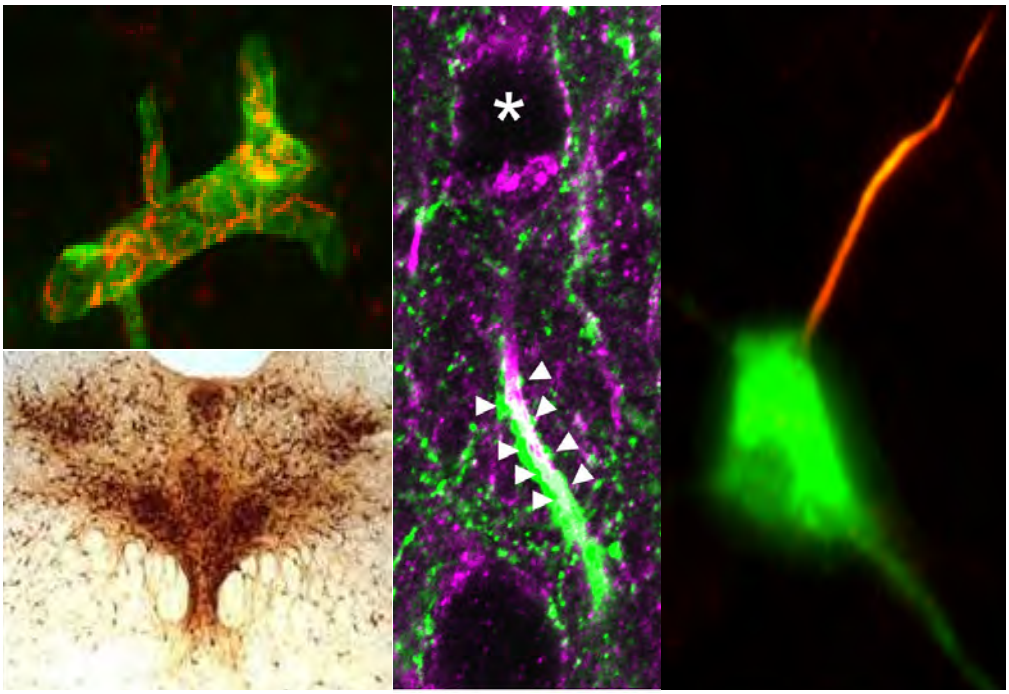
10TH ANNUAL MEETING OF THE

FRONT RANGE NEUROSCIENCE GROUP



November 28, 2012
Hilton Fort Collins

Annual Meeting: November 28, 2012
Hilton Fort Collins
10:00am – 6:30pm

PROGRAM

10:30-noon – Data Blitzing "NEW"

Noon-3pm – Lunch, Posters, Vendors!
12:00-1:30 ODD
1:30-3:00 EVEN

3-4pm – Award Winning Student presentations
Krystle Frahm (CSU)
Xinjun Wang (Univ Wyoming)
Nina Donner (CU Boulder)
Elizabeth Akin (CSU)

4:00-4:30pm – Coffee break, stretch

4:30-5:30pm –Keynote:

Dr. Stan Watson, MD, PhD, University of Michigan
Title: Postmortem Studies of Severe Mental Illness: Genomic and Anatomical Observations

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

SfN http://FRNG.colostate.eduSfN

Keynote Speaker

Dr. Stan Watson, MD, PhD
University of Michigan

Title: Postmortem Studies of Severe Mental Illness: Genomic and Anatomical Observations

The laboratory focuses on CNS circuits and cellular systems that participate and regulate states in the brains of individuals with severe mental illness. Using a variety of molecular, anatomical, behavioral and pharmacological approaches, his lab studies key circuits and molecules of interests.

from AllPsychologyCareers.com

Acknowledgements:

Cover Page: Designed by Christina Dennison
Scientific images provided *by Krystle Frahm (CSU), Xinjun Wang (Univ Wyoming), Nina Donner (UC Boulder) and Elizabeth Akin (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 (a division of DSM), Olympus America, Fisher Scientific, The Jackson Laboratory. 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 Jessica Veal 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 finally the parent Society for Neuroscience.

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, Christina Dennison, and Mallory Shields from CSU, Dori R. Pitynski and Colleen M. Cassidy from Univ Wyoming, Emily Aurand and Tara Martin from UC Anschutz Health Science Campus, Nina Donner and Jessica Babb from UC-Boulder and Josie Gray from DU. And additionally to Erin Bisenius, Brett Beal, and Sara Neys and the first year MCIN students Kristen and Sarah for helping with attendee registration.

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

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, Mark Thomas and Stuart Tobet.

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

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Your Morning DATA BLITZ . . . What is new in the Front Range?

Joan C. King, PhD, Emerita Professor, Anatomy & Cell Biology, Tufts University, Currently CEO Beyond-Success.com, "**New, Neuroscience, and Professional Development**"

Nancy Lorenzon, PhD, Assistant Professor, Biological Sciences, University of Denver: "**What's new at DU**".

Michael V. Baratta, PhD, Postdoctoral Fellow, Institute for Behavioral Genetics, University of Colorado Boulder, PI: Don Cooper: "**Any colour you like: novel tools for understanding resilience**"

Jeffrey P. Smith, PhD Associate Professor of Biology: Director, Master of Science Program for Biology Colorado State University-Pueblo: "**Regulation of monocarboxylic acid transport in the neurovascular unit: Importance for learning, memory and brain diseases**"

Glen Telling, PhD, Director, Prion Research Center, Department of Microbiology, Infectious Disease, & Pathology, Colorado State University: "**Prions, prions, prions**".

Travis Brown, PhD, School of Pharmacy, Graduate Neuroscience Program, Univ Wyoming: "**Interleukin-1 beta potentiates glycinergic synapses on lamina II neurons in the dorsal horn of the spinal cord**"

Jeremy R. Reynolds, PhD, Assistant Professor, Cognitive and Developmental Cognitive Neuroscience, University of Denver: "**Understanding how people perform goal- directed behaviors**".

Baskaran Thyagarajan, M.Pharm., PhD, School of Pharmacy, Graduate Neuroscience Program, Univ Wyoming: "**TRP Channels: Truly Remarkable Proteins in Physiology and Disease**."

Abby Person, PhD, Department of Physiology and Biophysics, UC Anschutz: "**Dissecting a corollary discharge pathway in the mammalian cerebellum**"

Amy Navratil, PhD, Department of Zoology and Physiology, Univ Wyoming: "**Brain to pituitary to world: keys to biological plasticity**"

Huntington Potter, PhD, Department of Neurology and the Linda Crnic Institute for Down Syndrome, UC Anschutz: "**Alzheimer's disease studies move from Florida to Colorado**".

Mark Stetter, DVM, Dean, College of Veterinary Medicine and Biomedical Sciences, Colorado State University: "**From Disney to CSU – The New Front Range Dean**".

Kelsey Poos-Benson and Brieanna Krueger, Neuroscience Program, Regis College: **Undergraduate neuroscience in the Front Range**.

ORAL ABSTRACT SESSION:

1. **Prenatal dexamethasone impacts the blood vessels within the paraventricular nucleus of the hypothalamus.**
   Krystle A. Frahm[1,2,3], Stuart A. Tobet[1,2,4]. From [1]Cell & Molecular Biology, [2]Department of Biomedical Science, [3]Program in Molecular, Cell and Integrative Neurosciences, [4]School of Biomedical Engineering, Colorado State University, Fort Collins, CO.

2. **Key role of axo-axonic innervations within olfactory cortex.**
   Xinjun Wang, Qian-Quan Sun. From the Neuroscience program, Department of Zoology and Physiology, University of Wyoming, Laramie, WY.

3. **Chronic glucocorticoid intake increases basal- and stress-induced tryptophan hydroxylase activity in anxiety-, sleep/wake-, and memory-related serotonergic systems.**
   Nina C. Donner, Philip H. Siebler, Sofia Mani, Christopher A. Lowry. From the University of Colorado at Boulder, Boulder, CO.

4. **Single-particle tracking of Nav1.6 suggests a novel anchoring mechanism and demonstrates direct trafficking to the AIS**
   Elizabeth J. Akin[1], Aubrey V. Weigel[2], Diego Krapf[2,4], and Michael M. Tamkun[1,3]
   From the [1]Department of Biomedical Sciences, [2]School of Biomedical Engineering, [3]Department of Biochemistry and Molecular Biology, [4]Department of Electrical and Computer Engineering, Colorado State University, Fort Collins, CO.

ORAL ABSTRACTS

Krystle A. Frahm, Stuart A. Tobet

Prenatal dexamethasone impacts the blood vessels within the paraventricular nucleus of the hypothalamus.

The Paraventricular Nucleus of the Hypothalamus (PVN) is a dense collection of neurons that play key roles in maintaining homeostasis and initiating stress responses. It is also characterized by a dense matrix of blood vessels compared to surrounding brain regions. The PVN has been implicated in mood disorders such as depression in which there is disruption of the hypothalamic-pituitary-adrenal axis known to be important in stress responses. Glucocorticoids have shown to alter the neural circuitry within the PVN but whether the blood vessels are also impacted has yet to be determined. Therefore, we investigated whether glucocorticoid signaling might also regulate the unique vasculature within the PVN. Pregnant Tie2-GFP transgenic mice on an FVB background were injected with 0.1mg/kg/day of dexamethasone or vehicle during embryonic days (E) 11-17. We have recently shown that the blood vessel pattern in the PVN is similar to surrounding brain regions until postnatal day 8 (P8). From P12 to P20 there is a dramatic vascular remodeling that leads to a dense vascular pattern by weaning that is maintained into adulthood (Frahm et al., 2012). Therefore, on P20, brains

were either immersed in 4% paraformaldehyde (0.1M PB, pH 7.4) overnight or perfused transcardially with heparin PBS containing fluorescein isothiocyanate (FITC) followed by 4% paraformaldehyde. Serial 50μm sections were cut using a vibrating microtome and collected through the entire PVN. Blood vessels were visualized by immunoreactive platelet endothelial cell adhesion molecule (PECAM) and pericytes as key components of the blood-brain barrier (BBB) were visualized using immunoreactive desmin. Images containing an ROI encompassing central portions of the PVN were analyzed from rostral to caudal. For blood vessel density, results showed significant decreases in blood vessel length and total immunoreactivity in dex- compared to vehicle-treated mice across the entire PVN ($p < 0.05$) and in the rostral and mid PVN regions particularly ($p < 0.01$). For branch points, there was a significant decrease in the rostral and mid regions of the PVN in dex-treated compared to vehicle-treated mice ($p < 0.05$). To investigate BBB competency, leakage of FITC from perfused blood vessels was determined using confocal microscopy. We found PVN-specific increased leakage in dex-treated mice compared to vehicle-treated ($p < 0.05$). We also found a change in the cellular composition in the PVN with dex-treated mice having increased desmin-immunoreactivity compared to vehicle-treated mice ($p < 0.05$), which has been shown to indicate vascular dysfunction. These results show that the overall blood vessel pattern and composition differs in dex-treated compared to vehicle-treated mice. Collectively these changes provide insight into the long-term functional consequences observed after prenatal dex-exposure. Overall, altered vascularity within the PVN may impair its neurons' ability to provide proper responses or feedback resulting in dysfunction. In general, a novel mechanism for fetal antecedent programming that may influence adult disorders may be through alterations in the functioning of neurovascular units.

Xinjun Wang, Qian-Quan Sun

Key role of axo-axonic innervations within olfactory cortex

Chandelier cells are characterized by their fast-spiking firing pattern, expression of parvalbumin and unique innervations onto the axon initial segments of excitatory neurons (axo-axonic innervations, AAIs). Due to the strategic location of axon initial segments, chandelier neurons are believed to provide strong control over neural outputs and further, contribute to neural oscillation. So far, the function of AAIs remains obscure due to the lack of specific cellular markers and the rarity of these cells.

In this study, the olfactory cortex of mice was used to investigate the anatomy and function of AAIs with a goal to gain insights into the function of chandelier cells in olfactory information processing. For the first time, we provide a thorough characterization of morphology and molecular composition of AAIs (Wang and Sun, 2012). GAT-1 (GABA Transporter 1) and Ankyrin-G were used as presynaptic and postsynaptic markers, respectively. We determined that the following presynaptic and postsynaptic proteins were expressed at AAIs: gephyrin, NKCC1 (Na-K-Cl Cotransporter-1), GABAAα1 (GABAA receptor α1), PV (Parvalbumin), VGAT (Vesicular GABA Transporter) and GAD67 (glutamic acid decarboxylase67). Next, in vitro slice electrophysiology combined with optogenetic tools was employed to study the physiological features and functions of AAIs. VGAT-ChR2 mice and laser mapping technology allowed us to optically activate chandelier innervations according to their unique locations. To detect true chloride homeostasis, we also took advantage of gramicidin perforated patch recordings. Further, a novel axon lesion slice method was utilized to study the effect of the elimination of chandelier innervations.

In summary, our results demonstrated for the first time that in the PC of mice, highest densities of chandelier innervations were found among all cortices. Functionally, fast-rising and large hyper-polarizations onto excitatory neurons were detected upon the activation of chandelier innervations. These inhibitory inputs provided a profound and long-lasting inhibitory effect on the excitability of postsynaptic glutamatergic neurons.

Nina C. Donner, Philip H. Siebler, Sofia Mani, Christopher A. Lowry.

Chronic glucocorticoid intake increases basal- and stress-induced tryptophan hydroxylase activity in anxiety-, sleep/wake-, and memory-related serotonergic systems

Major depressive disorder is linked to both disruption of hypothalamic-pituitary-adrenal (HPA) axis function and dysfunction of brain serotonin (5-hydroxytryptamine, 5-HT) systems. However, causal relationships leading to dysregulation of these systems are still unclear. To test the hypothesis that chronic glucocorticoid (GC) exposure, without external stressors per se, alters both basal and stress-induced activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme for 5-HT synthesis, we treated adrenal-intact adult, male rats with 100 µg/ml corticosterone (CORT) or vehicle (0.45% 2-hydroxypropyl-β-cyclodextrin) via the drinking water for 21 days. We monitored weight gain and diurnal food and water consumption, and assessed the rats' emotionality in the elevated plus-maze (EPM) and forced swim tests (FST) after two weeks. On day 21, all rats were injected with 100 mg/kg NSD-1015, an inhibitor of aromatic amino acid decarboxylase, to block the conversion of 5-hydroxytryptophan (5-HTP) to 5-HT. Following the injection, rats were either exposed to acoustic startle (AS) for 30 min or remained in home cages until rapid decapitation. Post mortem, we assessed ex vivo adrenal functionality in a tissue culture assay, and measured 5-HTP accumulation, a read-out of TPH activity, in the median raphe nucleus (MnR) and dorsal raphe nucleus (DR) subregions, and in anxiety-related forebrain target areas, using high-pressure-liquid chromatography. Chronic CORT intake caused reduced weight gain, anxiety-like behavior on the EPM, increased immobility in the FST, an increased AS response, and adrenal insufficiency. The diurnal pattern and the amount of food and water intake remained unchanged. Most importantly, chronic CORT elevated both basal and AS-induced TPH activity in the dorsal (DRD) and ventral DR (DRV), as well as basal TPH activity in the MnR. In the caudal DR (DRC), the infralimbic cortex (IL), the dorsal cornu ammonis region of the hippocampus (dCA1) and the caudal pontine reticular formation (PnC), AS elevated TPH activity only in CORT-, but not in vehicle-treated rats, indicating a permissive effect of chronic CORT. The increase of TPH activity in the PnC was strongly correlated with the magnitude of the AS response. Our results are novel, and suggest that GCs exert their chronic actions by creating either a basal hyperactivity or a hypersensitivity to an acute stressor in those serotonergic circuitries that modulate anxiety-related behavior (DRC, DRD, DRV, IL) as well as sleep/wake function (MnR, PnC) and learning and memory (dCA1). This study was funded by an HHMI individual grant (SM) and by the NIMH grant R01MH086539 (CAL).

Elizabeth J. Akin, Aubrey V. Weigel, Diego Krapf, Michael M. Tamkun

Single-particle tracking of Nav1.6 suggests a novel anchoring mechanism and demonstrates direct trafficking to the AIS

Voltage-gated sodium channels are responsible for the initiation of action potentials in excitable cells. These channels are highly concentrated at the axon initial segment (AIS) of neurons due to their interactions with

ankyrin-G. This interaction is mediated by a 9 amino acid sequence, termed the Ankyrin Binding Motif (ABM) present on the II-III linker. In order to study the dynamics of sodium channels in living neurons in real time, we created a fluorescently labeled Nav1.6 protein with an extracellular tag (biotin acceptor domain). We used single-particle tracking of channels labeled with streptavidin conjugated quantum dots (QDs) and/or Alexa594 (A594) to directly compare the mobility of Nav1.6 channels localized to the AIS and somatodendritic compartments of 8DIV hippocampal neurons. We observed two populations of Nav1.6 channels, a small mobile population and a much larger immobile population. To determine the role of ankyrin-G binding in the diffusion of the full-length sodium channel, we deleted the ABM from the Nav1.6 construct. As expected, this mutant channel did not concentrate at the AIS and instead was localized throughout the soma and processes, based on both GFP fluorescence and labeling of surface channels using A594. Single-particle tracking of the mutant channels revealed that the majority of these channels are also immobile in the plasma membrane of the soma and dendrites. This suggests that although binding to ankyrin-G is necessary and sufficient for Nav1.6 to localize to the AIS, a different mechanism is responsible for the localization and membrane dynamics in the somatodendritic region of hippocampal neurons. Using A594 to label newly inserted channels, we observe that channels localize to the AIS via direct trafficking, rather than diffusion trapping. This is consistent with the idea that the majority of channels on the neuronal surface have low mobility.

POSTER PRESENTATIONS

Cognition and Behavior

1) Serotonin as a mediator of aggression in the stalk-eyed fly. AN Bubak, JG Swallow, KJ Renner.

2) A comparison of EEG systems for use with brain-computer interfaces in home environments. BK Cabral, EM Forney, CW Anderson, PL Davies, WJ Gavin.

3) Dissociating the functional role of the dorsal and ventral subiculum using a novel Go/No-go contextual tone discrimination task in the rodent. SD Dolzani, S Nakamura, DC Cooper.

4) An fMRI investigation of the accumulation of information for categorization. G Fan, K Braunlich, C Seger.

5) The controllability of stress determines phosphorylation of extracellular signal-regulated kinases 1 and 2 in dorsal striatum and prefrontal cortex. JG Flyer, JP Christianson, LR Watkins, SF Maier.

6) Non-Invasive brain-computer interfaces using echo state networks. EM Forney, CW Anderson, WJ Gavin, PL Davies, BK Cabral.

7) ERP correlates of recognition without identification across real-world visual stimuli. SR Staley, AJ Ryals, SJ Leonard, MJ Cowen, WJ Maher, TJ Hawkins, AM Cleary.

8) Fibroblast Growth Factor 8 deficiency impacts anxiety-like behavior in response to restraint stress in mice.LR Brooks, CL Enix, SC Rich, HL Pals, CA Lowry, P-S Tsai.

9) Enhanced cocaine self-administration in mice with conditional knockdown of forebrain TRPC5. MB Pomrenze, MV Baratta, KC Rasmus, BA Cadle, L Birnbaumer, DC Cooper.

Development

10) Effects of enzymatically inactive recombinant botulinum neurotoxin type A at the mouse neuromuscular junctions. P Baskaran, T Lehmann, E Topchiy, N Thirunavukkarasu, S Deshpande, S Cai, BR Singh, B Thyagarajan.

11) Maturation of intrinsic properties in the optic tectum of the Xenopus tadpole. AS Hamodi, KG Pratt.

12) The role of the neurokinin 3 receptor in neurite outgrowth. CJ Hoekstra, EW Kinney-Lang, FW Flynn.

13) Connexin 35b in zebrafish spinal cord development. TC Martin, AB Ribera.

14) Embryonic GABAB receptor blockade alters cell migration, adult hypothalamic structure, HPA axis activation, and anxiety- and depression-like behaviors in mice. C Nash, M Steigerwald, M Stratton, T Budefeld, D Carbone, R Handa, G Majdic, S Tobet.

15) Role of the prion protein in olfactory system development. LE Parrie, RA Bessen.

16) Protocadherin10a acts downstream of prdm1a in The formation of neural crest-derived pigment cells. CC Rossi, J Williams, L Hernandez, KB Artinger.

17) Characterization of neonatal seizures in an animal model of hypoxic-ischemic encephalopathy. D Sampath, A White, Y Raol.

18) FMRP- and miRNA-mediated regulation of synapse structure in drosophila. BA Symmes, L Rozeboom, SA Barbee.

19) Lipid microdomains regulate neuronal endocytic mechanisms during development X Tang, P Baskaran, K Hognason, J Potian, J McArdle, B Thyagarajan.

Disorders of the Nervous System

20) Neural stem cells and hydrogels: enhancing neural tissue engineering potential. ER Aurand, J Wagner, C Lanning, KB Bjugstad.

21) Augmentation of mitochondrial glutathione transport renders a motor neuron cell line resistant to oxidative stress. S Brock, H Wilkins, DA Linseman.

22) Dietary docosahexaenoic acid (DHA) improves behavioral and biomarker outcomes when provided before or after experimental diffuse brain injury. CM Butt, J Lifshitz, J Jones, N Salem, Jr., JR Pauly.

23) c-Myb and its possible role in schizophrenia. CJ Cabrera Montalvo, JA Stitzel.

24) Early-life iron intake modulates late-life outcomes in Huntington's disease mice. J Chen, J Fox, J Moline, J Duce, I Volitakis, A Bush.

25) Toll Like Receptor 4 (TLR4) antagonism suppresses cocaine reward. TA Cochran, AL Northcutt, EL Galer, ME Haas, MR Hutchinson, CE O'Neill, X Wang, NE Miles, J Amat, SF Maier, RK Bachtell, KC Rice, LR Watkins.

26) SK Grotewold, V Wall, C Hayter, A Bowman, D Goodell, ST Bland.

27) Toll Like Receptor 4 antagonism attenuates cocaine and methamphetamine induced dopamine increases in the nucleus accumbens. ME Haas, AL Northcutt, EL Galer, TA Cochran, MR Hutchinson, X Wang, NE Miles, SF Maier, KC Rice, LR Watkins.

28) Selenium supplementation is neuroprotective in mouse Huntington's disease and normalizes liver selenium. Z Lu, J Chen, E Marks, J Moline, L Barrows, M Raisbeck, JH Fox.

29) Does inhibition of the 5-LO leukotriene synthesis pathway with MK-886 ameliorate disruption of the BBB and edema following fluid percussion injury? P Šerbredzija, CE Corser-Jensen, DJ Goodell, RC Murphy, and KA Heidenreich.

30) Dysregulation of Rho or Rac elicits death of NSC34 cells and activation of these GTPases is altered in G93A mSOD1 mice. TR Stankiewicz, RJ Bouchard, DA Linseman.

31) Procyanidin B2 protects neurons from oxidative, nitrosative, and excitotoxic stress. TC Sutcliffe, AN Winter, DA Linseman.

32) Neuroprotective efficacy and pharmacokinetics of novel para-phenyl substituted diindolylmethanes in a model of Parkinson's disease. B Trout, J Miller, R Hansen, S Safe, D Gustafson, R Tjalkens.

33) Mitochondrial glutathione transport is a key determinant of neuronal susceptibility to oxidative and nitrosative stress. HM Wilkens, D Kirchhof, E Manning, JW Joseph, DA Linseman.

34) Chemical basis for the disparate neuroprotective effects of the anthocyanins, callistephin and kuromanin, against nitrosative stress. AN Winter, EK Ross, DA Linseman.

Neural Excitability, Synapse and Glia

35) Structure and dynamics of the synaptophysin/VAMP2 complex. DJ Adams, CP Arthur, MHB Stowell.

36) Uncovering roles for fractalkine in CD4+ T cell migration into the central nervous system in patients with relapsing remitting multiple sclerosis. K Blauth, X Zhang, K Marcus, D Bruce, M Chopra, L Troiani, Si Markovic-Plese.

37) Arterial smooth muscle oxidant and calcium microdomain signaling. NL Chaplin, MM Frey, AM Fresquez, GC Amberg.

38) Endoplasmic reticulum/plasma membrane junctions function as membrane protein trafficking hubs at the neuronal perikaryon. PD Fox, CJ Haberkorn, AV Wiegel, EJ Akin, MJ Kennedy, D Krapf, MM Tamkun.

39) μ-opioid receptor mediated modulation of intrinsically photosensitive retinal ganglion cells. SK Gallagher, J Vigh

40) Monocarboxylate transporter 1 trafficking and regulation by cAMP analogs in rat brain endothelial cells. N Lavoy, L Li, MJ Velasquez, C Soole AL Uhernik, JP Smith.

41) Dopamine receptor stimulation alters intrinsic resonance properties in Layer 5 pyramidal neurons in mouse medial prefrontal cortex. J Leyrer, J Erickson, M Spindle, M Thomas.

42) Using light response to trigger exocytosis from bipolar cell terminals. M Lipin, J Vigh.

43) NF-kB activation in the hippocampus during multiple sub-threshold exposures to seizuregenic compounds. JA Miller, KA Sullivan, YH Raol, M Patel, RA Bialecki, RB Tjalkens.

44) The role of the C_2A domain of synaptotagmin in asynchronous release. M Shields, N Reist.

45) Activity-dependent plasticity in the retina. RE Tooker, M Lipin, SK Gallagher, J Bramley, E Rozsa, J Vigh.

Neuroendocrine

46) Mismatch of tachykinin innervation and receptors in the magnocellular portion of the paraventricular hypothalamus in rat. CR Brown, FW Flynn.

47) Clock gene mRNA expression in the paraventricular nucleus of the hypothalamus and the prefrontal cortex in male and female rats. LE Chun, L Woodruff, LR Hinds, RL Spencer.

48) Local L-type calcium channel signaling in αT3-1 cells. AK Dang, NL Chaplin, C Magee, Di Murtazina, CM Clay, GC Amberg.

49) Microfluidic strategy for spatiotemporally resolved molecular sampling from live organ slices. C Eitel, J Wydallis, V Pauna, C Eslinger, M Mensack, DS Dandy, CS Henry, S Tobet.

50) Visualizing cell populations with harmonic generation microscopy in the region of the paraventricular nucleus. JJ Field, C Eitel, KA Frahm, SA Tobet, RA Bartels.

51) Predation pressure alters stress physiology in guppies. EK Fischer, RM Harris, HA Hofmann, KL Hoke.

52) Localization of a gonadotropin-releasing hormone like-molecule in a gastropod mollusk, Aplysia californica. L Jung, SI Kavanaugh, P-S Tsai.

53) Chronic glucocorticoid intake abolishes the diurnal pattern of slc6a4 and htr1a mRNA expression in anxiety-related serotonergic systems. S Mani, NC Donner, AJ Matti, CA Lowry.

54) Fibroblastic growth factor signaling deficiencies exerted variable age-dependent effects on SCN VIP neurons of male mice. AV Miller, SI Kavanaugh, MA Basse, SD King, PS Tsai.

55) Endocannabinoid signaling as an intrinsic component of the circuits mediating adaptive responses to repeated stress exposures. RJ Newsom, CV Masini, TJ Nyhuis, HE Day, S Campeau.

56) Reversible inactivation of the rostral raphe pallidus disrupts acute autonomic responses but not habituation to repeated loud noise stress exposures in rats. CV Masini, TJ Nyhuis, HEW Day, S Campeau.

57) Reversible inactivation of a subregion of the posterior hypothalamus disrupts HPA axis habituation to repeated stress exposures in rats. TJ Nyhuis, CV Masini, RJ Newsom, HEW. Day, S Campeau

58) Corticosterone treatment immediately after stress onset produces a delayed negative feedback effect on HPA axis activity. C Osterlund, M Rodriguez, R Spencer.

59) Characterization of glucocorticoid modulation of clock gene oscillation in the frontal cortex. L Woodruff, LE Chun, M Girotti, LR Hinds, RE Ramsey, C Osterlund, RL Spencer.

Sensory and Motor Systems

60) Applying mutual information theory to the auditory system. K Anbuhl, D Tollin.

61) Peripheral anatomy of two classes of slowly adapting vibrissal sinus hair follicle afferents. CM Cassidy, CJ Woodbury.

62) Peripheral color vision can be better than foveal color vision. JK Opper, ND Douda, VJ Volbrecht, JL Nerger.

63) The Nuclei of the lateral lemniscus: Cytoarchitecture and connectivity with the inferior c colliculus. DR Pitynskil, ZM Fuzessery.

64) Differential distribution of AgRP and POMC in taste buds of mice. SH Monahan, ST Hentges, LM Stone.

65) Cholesterol depletion activates TRPV1. B Surenkhuu, P Baskaran, S Clark, B Culver, B Thyagarajan.

66) Activity in the mouse pedunculopontine tegmental nucleus correlates with action selection and execution. JA Thompson, Gidon Felsen.

67) Balancing excitation and inhibition within olfactory bulb glomeruli. JD Zak, DH Gire, NE Schoppa.

Teaching

68) Educational opportunities for CSU students and local high school students during Brain Awareness Week. LS Baker, LM Stone

69) From the pupil's perspective: A look at the benefits outreach presentations offer pre-professional students. AH Beach, LL Leach, TR Clapp.

70) Analyzing neuroscience educational approaches and success. M Mesiha, S Hentges.

71) The use of non-prescribed Adderall and Ritalin in a collegiate setting. ML Miller, TR Clapp.

72) The case study: Figuring out how to figure it out. A Vaudreuil, TR Clapp.

ABSTRACTS

Cognition and Behavior

1) **Serotonin as a Mediator of Aggression in the Stalk-Eyed Fly.**
AN Bubak, JG Swallow, KJ Renner. From the University of Colorado-Denver, Denver, CO and University of South Dakota, Vermillion, SD.
The outcome of behavioral interactions between organisms can have significant fitness implications. Therefore, it is of great theoretical and practical importance to understand the mechanisms that modify different behavioral syndromes. Changes in central monoamines, such as serotonin (5-HT) may contribute to modifying the expression of behavioral syndromes in invertebrates. In several invertebrate groups, neural 5-HT has been linked to heightened aggression and conflict escalation. The male stalk-eyed fly (Teleopsis dalmanni) competes with conspecifics daily over access to resources such as food and mates. Because encounters escalate in a stereotypical manner, stalk-eyed flies provide an excellent model system to study behavioral syndromes. We hypothesized that pharmacological augmentation of brain 5-HT would increase stereotypic behavioral escalation and the probability of winning a conflict over food. To address this hypothesis, we first determined that administration of the 5-HT precursor, 5-hydroxytryptophan (5-HTP) in food results in dose-dependent increases in brain 5-HT levels using high performance liquid chromatography with electrochemical detection (HPLC). In the second experiment, size-matched male 5-HTP-treated and untreated flies were placed in a ten-minute forced-fight paradigm and their aggressive behaviors were scored. Individuals pretreated with 5-HTP had higher brain 5-HT levels ($P<0.001$) and a higher probability of winning the contests ($P<0.005$). Since pretreatment with 5-HTP did not significantly alter other brain monoamines, these results implicate 5-HT as a potential mediator of aggression in these organisms and may play a role in determining reproductive success and resource attainment. Supported by NSF grants IOS 0921874 (KJR) and IOB 0448060 (JGS).

2) **A Comparison of EEG Systems for use with Brain-Computer Interfaces in Home Environments.**
BK Cabral, EM Forney, CW Anderson, PL Davies, WJ Gavin. From the Department of Computer Science, the Department of Human Development and Family Studies, and the Department of Occupational Therapy, Colorado State University, Fort Collins, CO.
Brain-Computer Interfaces (BCI) establish a direct channel of communication between a user's brain and a computer system. Since BCI allow users to operate computerized devices only by manipulating their mental state, they are of interest to people with severe motor impairments. Although a number of research groups have demonstrated non-invasive BCI based on electroencephalography (EEG), these systems are often evaluated only in laboratory settings and with subjects that do not have disabilities. We seek to evaluate BCI under real-world conditions and determine the properties that EEG systems should possess in order to be suitable for use in BCI. Specifically, we investigate the performance of a BCI system known as the serial P300 speller. A P300 is a positive voltage deflection in EEG found 300ms following the presentation of a "rare but expected" stimulus. A serial P300 speller operates by presenting a series of flashing characters to the user and searching for a P300 following the character the user was attending to. EEG data was recorded from 16 subjects. Nine subjects did not have disabilities and recording took place in a laboratory setting. Seven subjects had severe motor impairments and recording took place in their home environments. Each subject participated in three sessions with a different EEG system used during each session. The three EEG systems vary significantly with respect to cost, portability, ease-of-use and signal resolution. The subsequent analysis produced a number of interesting results. First, a spectral analysis has revealed that there are higher levels of both 60Hz and broadband noise present in more realistic settings with the largest differences being observable in the lower-end systems. An analysis of averaged responses demonstrates that all three systems can be used to observe P300 waveforms, although it is clearly more difficult in real-world environments. Additionally, the lower-end systems have more difficulty resolving finer peaks in these waveforms. Further analysis is required in order to investigate the impact that these differences may have on the performance of BCI.
EXPERTISE: encephalography, brain-computer interfacing, pattern analysis

3) **Dissociating the functional role of the dorsal and ventral subiculum using a novel Go/No-go contextual tone discrimination task in the rodent.**

SD Dolzani, S Nakamura, DC Cooper. From the Department of Psychology and Neuroscience, University of Colorado Boulder, Boulder, CO.

Innovative molecular tools allow neuroscientists to study neural circuitry associated with specific behaviors. Consequently, behavioral methods must be developed to interface with these new molecular tools in order to identify the causal elements underlying behavior and decision-making processes. Here we present an apparatus and protocol for a novel Go/No-Go behavioral paradigm to study the brain attention and motivation/reward circuitry in awake, head-restrained rodents. This experimental setup allows: (1) Pharmacological and viral manipulation of various brain regions via targeted guide cannula and ; (2) Optogenetic cell-type specific activation and silencing. The behavioral paradigm consists of three events. The subject initiates a trial by lever pressing in response to a respective Go or No-go tone. Following a delay period after initiation of the trial, the subject is presented with a challenge period during which they are required to lever-press (Go) or withhold from lever-pressing (No-go) during a white-noise presentation. After successfully responding during the challenge period, a final tone of the same frequency as the initiating tone is presented and sucrose delivery is contingent on lever pressing during this tone. This paradigm allows researchers to study and manipulate components of behavior, such as motivation, impulsivity, and reward-related working memory during an ongoing operant behavioral task without interference from non task-related behaviors. Furthermore, using this newly developed Go/No-go task, we investigated functional roles of the dorsal and ventral subiculum, which are critical but under-investigated primary output regions of the hippocampus that have been implicated in reward-related working memory. Pharmacologically inactivating the dorsal subiculum using muscimol increased incorrect responding during the challenge period of No-go trials, while inactivation of the ventral subiculum decreased correct responding during the challenge period of Go trials. In order to further dissect the contribution of the dorsal and ventral subiculum to behavioral task performance, we utilized temporally precise optogenetic silencing in halorhodopsin-expressing rats.

EXPERTISE: Optogenetics, IHC, behavioral testing, cytology, drug SA, surgical procedures, stereotaxic cannula implantation, virally mediated gene introduction

4) **An fMRI investigation of the accumulation of information for categorization.**

G Fan, K Braunlich, C Seger. From the Department of Psychology, Colorado State University, Fort Collins, CO.

The goal of the present study was to localize regions associated with perceptual processing, decision-making and the accumulation of weighted information indicating category membership. Prior to scanning, participants learned to categorize individual icons into one of two categories via trial and error. While some exemplars were highly predictive of a particular category (i.e., the correct response was the same on 90% of the trials), other exemplars had lower category weights (80%, 65% or 50%). In the fMRI task, an array of icons was presented, one-by-one, at a rate of 2 s per icon. Participants had to decide what category the overall array belonged to on the basis of the preponderance of evidence provided by the icons and were instructed to make a categorization decision as soon as they believed they had sufficient information. Participants won points for correct responses and lost points for incorrect responses. To encourage participants to respond both quickly and accurately, the magnitude of gains and losses was greater for responses made on the basis of fewer icons. By manipulating the order in which icons were presented, we were able to influence when participants made their decisions. Icons were presented in three accumulation orders: slow (information accumulated slowly towards one category), fast, and “switch” (information accumulated slowly towards one category, and then towards the other). We also controlled when information accumulation began; on late accumulation trials, several non-informative exemplars (50% category weight) were presented prior to the informative exemplars. Neuroimaging results highlight regions that were associated with the perceptual processing of icons and were indiscriminate to differing category weights, regions that were associated with decision processes, and regions that were sensitive to accumulation of weighted information about category membership.

EXPERTISE: behavior testing

5) **The controllability of stress determines phosphorylation of extracellular signal-regulated kinases 1 and 2 in dorsal striatum and prefrontal cortex.**

JG Flyer, JP Christianson, LR Watkins, SF Maier. From the Department of Psychology and Neuroscience and Center for Neuroscience, University of Colorado Boulder, Boulder, CO.

Learning to use action-outcome contingencies, a form of instrumental learning, involves a frontostriatal circuit including the dorsal striatum and ventromedial prefrontal cortex. Thus it follows that learning an instrumental

response to escape a stressor may involve this circuit. We have demonstrated that escapable (controllable) stress recruits both the dorsal striatum and the prefrontal cortex (PFC), making them putative sites for neuroplasticity. However, the molecular mediators of plasticity in this paradigm remain unknown. Maintenance of long-term potentiation and consolidation of memory in vivo require the mitogen-activated protein kinases (MAPK). Specifically, extracellular-regulated kinase (ERK, also known as MAPK1) is critical to memory. Therefore the current study tested the hypothesis that exposure to escapable stress would lead to ERK phosphorylation in the PFC and dorsal striatum. Subjects (male Sprague-Dawley rats) were exposed to 80 trials of either controllable or uncontrollable tailshock stress, and sacrificed either immediately or 1 hr post treatment. The ratio of phosphorylated ERK to total ERK was determined in micropunches taken from prelimbic or infralimbic cortex, dorsomedial or dorsolateral striatum and basolateral amygdala by Western blot. Results indicate that controllable stress increased ERK phosphorylation in the prefrontal cortex and dorsal striatum. At these timepoints, no effects of stress were observed in the basolateral amygdala. ERKs appear to be mediators of the effects of controllable stress on subsequent behavior.
EXPERTISE: Behavior testing, Western blot, stereotaxic surgeries, immunohistochemistry, microscopy, real time qPCR, PCR etc.

6) **Non-Invasive Brain-Computer Interfaces using Echo State Networks.**
EM Forney, CW Anderson, WJ Gavin, PL Davies, BK Cabral. From the Department of Computer Science, the Department of Human Development and Family Studies, and the Department of Occupational Therapy, Colorado State University, Fort Collins, CO.
A brain-computer interface (BCI) is a device that enables a user to communicate with a computer system by voluntarily altering their mental state. In one approach for constructing BCI, a user issues instructions to a computer by performing one of several mental tasks. Electroencephalography (EEG) can then be used to monitor brain activity while a machine learning algorithm finds patterns in the EEG that are unique to the mental task the user is performing. Using such a BCI in real time, a person might silently sing a song to move a mouse cursor to the left or perform a mathematical task to move the cursor to the right. We investigate the use of a novel algorithm for identifying which mental task a user is performing. This algorithm utilizes a type of artificial neural network known as an Echo State Network (ESN). First, ESN are trained to model EEG by forecasting the signal a single step ahead in time. We are able to demonstrate that ESN can produce signals similar to true EEG. Next, we train a separate ESN to model EEG produced while the subject performs each of several mental tasks. In this way, we have a separate ESN that is an expert at forecasting EEG from each task. Previously unseen EEG is labeled by applying each ESN and selecting the label associated with the model that produced the lowest forecasting error. Data was recorded from 14 subjects using a portable EEG system. Five subjects had severe motor impairments and recording took place in their homes. Nine subjects had no disabilities and recording took place in a laboratory environment. Each subject performed four mental tasks following a visual queue on a computer screen. Five ten-second trials were recorded from each subject for offline analysis. Classification accuracies as high as 65% correct when using all four tasks and as high as 95% for a two-task problem were achieved at two-second intervals. Although the users with disabilities did not perform as well as the users without disabilities, the difference is not statistically significant. Information transfer rates as high as 21 bits per minute were achieved, comparable with state-of-the-art BCI systems.
EXPERTISE: electroencephalography, brain-computer interfacing, pattern analysis

7) **ERP correlates of recognition without identification across real-world visual stimuli**
SR Staley, AJ Ryals, SJ Leonard, MJ Cowen, WJ Maher, TJ Hawkins, AM Cleary. From the Department of Psychology, Colorado State University.
The present study examined the electrophysiological correlates of the real-world-stimulus RWI effect with faces and scenes. In two experiments, participants judged photographs that had been filtered by a monochromatic noise filter while high-density EEG was being recorded. Experiment 1 consisted of filtered famous and non-famous faces, and Experiment 2 consisted of filtered famous and non-famous scenes. Participants rated familiarity and attempted to identify the face or scene for each filtered photograph. A final phase of each experiment consisted of presentation of the famous faces or scenes unfiltered in order to determine whether the subject would have known the famous face or scene had perception not been hindered through masking. A behavioral RWI effect was observed in both types of stimuli by which familiarity ratings were higher for filtered famous photographs that were later identified than for the filtered non-famous photographs.
Electrophysiological recordings revealed higher negativity at 140-190ms, consistent with the N170, for unidentified filtered non-famous faces than for unidentified filtered famous faces that were later identified when

unfiltered. In addition, an N300 component was observed whereby filtered famous scenes that were later identified when unfiltered evoked a higher negativity at 250-350 ms than did non-famous scenes. These findings implicate early processing of familiarity even before conscious recognition of the stimuli.

8) **Fibroblast Growth Factor 8 deficiency impacts anxiety-like behavior in response to restraint stress in mice.**

LR Brooks, CL Enix, SC Rich, HL Pals, CA Lowry, P-S Tsai. From the University of Colorado-Boulder, Boulder, CO.

Vulnerability to a number of psychiatric illnesses, including anxiety, may be neurodevelopmental in origin. Animal models suggest that serotonin (5-HT) neurons in the dorsal raphe nucleus (DR) are needed to establish functional stress- and anxiety- related circuitries, and environmental stressors can affect these circuits to precipitate anxiety-like behaviors. Fibroblast growth factor 8 (Fgf8) is critical for the development of 5-HT neurons, and its deficiency has previously been shown to lead to increased trait anxiety-like behavior in mice. However, it is unclear if the development of 5-HT neurons underlying stress- induced anxiety is also dependent on Fgf8 signaling. Given that stress is an important factor in the onset and expression of anxiety in humans, this study examined both stress-induced anxiety and 5-HT cell numbers in relevant DR subdivisions (dorsal, ventral, ventrolateral, caudal, interfascicular) in wildtype (WT) and Fgf8-deficient mice. WT and Fgf8-deficient mice were assigned to either a non-stressed or stressed group. Following one hour of no stress or restraint stress, adult Fgf8-deficient and WT mice were immediately tested for anxiety-like behavior in the elevated plus-maze (EPM). As expected, non-stressed WT mice spent significantly more time exploring the open arms compared to non-stressed Fgf8-deficient mice and their WT stressed counterparts. Surprisingly, stressed Fgf8-deficient mice spent significantly more time in the open arms than their non-stressed controls, suggesting that the stressed Fgf8 Het mice are less anxious than their non-stressed counterparts. There was no genotype difference in the stressed group. These findings suggest that stress impacts anxiety-like behavior differentially in WT and Fgf8-deficient mice. Neuroanatomical data support these findings by demonstrating decreases in both the number and density of neurons immunopositive for the serotonergic marker, tryptophan hydroxylase, in subregions of the DR associated with stress- and anxiety-like behaviors. These results expand our knowledge on the elements governing the development of specific subpopulations of 5-HT neurons, their functional integrity, and their relationship to stress-induced anxiety-related behavior. Support: NIH R01 HD042634, R01 MH086539.

9) **Enhanced cocaine self-administration in mice with conditional knockdown of forebrain TRPC5.**

MB Pomrenze[1], MV Baratta[1], KC Rasmus[1], BA Cadle[1], L Birnbaumer[2], DC Cooper[1]. From the [1]Institute for Behavioral Genetics, University of Colorado, Boulder, CO, [2]National Institute of Environmental Health Science, NIH, Research Triangle Park, NC.

Canonical transient receptor potential (TRPC) channels are a family of non-selective cation channels that play a crucial role in modulating neuronal excitability due to their involvement in intracellular Ca2+ regulation and dendritic arborization. The TRPC5 isoform a) is one of the two most prevalent TRPC channels in the adult rodent brain; b) is densely expressed in deep-layer pyramidal neurons in the prefrontal cortex (PFC); and c) modulates neuronal persistent activity necessary for working memory and attention. In order to evaluate the causal role of TRPC5 in motivation/reward-related behaviors, conditional knockdown of TRPC5 was achieved by crossing floxed trpc5 mice with mice that express Cre recombinase under the control of the αCamKII promoter, in which Cre transgene expression occurs postnatally and is restricted to excitatory neurons of the forebrain. TRPC5 KO mice and their wild-type (WT) littermates were trained to nose-poke for intravenous cocaine (0.75 mg/kg/infusion) during daily 3-h sessions under an FR-1 schedule of reinforcement without prior operant training. Both groups reached a stable level of responding within 5-7 days and subsequently exhibited similar responding to varying unit doses of cocaine (0.05, 0.1, 0.75, and 2.0 mg/kg/infusion). However, TRPC5 KO mice exhibited increased drug intake and an elevated rate of intake during the acquisition phase of cocaine self-administration compared to WT. The enhanced acquisition was not due to increased exploration or heightened sensitivity to the drug-paired cue. Breakpoints achieved in a progressive ratio schedule of reinforcement revealed an enhanced motivation to obtain cocaine in TRPC5 KO mice. These results suggest that TRPC5 channels in the PFC are important for regulating behavioral responses to cocaine and that reduced function and/or mutation in these channels may contribute to susceptibility for drug-seeking. Grant Support: RO1 DA24040 (DCC); T32 DA017637 (MVB); Z01 ES101684 (LB).

EXPERTISE: Optogenetics, viral-mediated gene delivery, drug self-administration, other behavior testing.

Development

10) **Effects of enzymatically inactive recombinant botulinum neurotoxin type A at the mouse neuromuscular junctions.**

P Baskaran[2], T Lehmann[3], E Topchiy[3], N Thirunavukkarasu[4], S Deshpande[2], S Cai[4], BR Singh[4], B Thyagarajan[1,2,5,*].From the [1]Neuroscience Program, [2]School of Pharmacy, [3]College of Health Sciences, and the [4]Dept. of Chemistry, College of Arts and Sciences at the University of Wyoming; [5]Dept. Chemistry and Biochemistry at the University of Massachusetts-Dartmouth, North Dartmouth, MA 02747-2300.
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Botulinum neurotoxin A (BoNT/A) is the most potent neurotoxin known to mankind. BoNT/A is also a potent biothreat agent and is designated by CDC as a Class A select agent. However, BoNT/A is used clinically for the treatment of several neurological and metabolic diseases. BoNT/A proteolyzes SNAP-25 at the motor nerve terminals and inhibits acetylcholine (ACh).

This results in the paralysis of innervating skeletal muscle. The long-lasting effects of BoNT/A underlie its therapeutic uses. However, nothing is known about the biological half-life of BoNT/A at the MNT. Further, the use of extremely low concentrations of BoNT/A makes it difficult to determine new cellular mechanisms and binding partners of BoNT/A in cellular systems. In order to address this, a non-toxic version of BoNT/A, designated as deactivated recombinant BoNT/A (DRBoNT/A) was developed and characterized. DRBoNT/A (150 ng) lacks the proteolytic activity (SNAP-25 cleavage) at concentrations as high as 45000 - folds compared to wild-type BoNT/A. Unlike BoNT/A injection (3.2 pg), injection of DRBoNT/A (150 ng) into mouse hind limbs failed to cause neuroparalysis as exhibited by the lack of inhibition of toe spread reflex (ability of the mouse to spread its hind limb toes), and inhibit ACh release at the MNT. In vitro experiments also demonstrate that DRBoNT/A uptake (at conc in 1000 –fold higher than wild type BoNT/A), internalization and localization at the presynaptic nerve terminals remained unaltered. Molecular dynamics analyses demonstrate that DRBoNT/A exhibits weak zinc binding ability. Collectively, we demonstrate that DRBoNT/A is non-toxic at the MNT. The recombinant deactivated BoNT/A can be used as a surrogate tool to understand the mechanism by which BoNT/A inhibits signal transduction mechanisms. Acknowledgment: 1001070G NIH/DHHS COBRE (B Thyagarajan).

EXPERTISE: Cell culture, immunocytochemistry, animal behavior, intracellular calcium imaging, and confocal microscopy.

11) **Maturation of intrinsic properties in the optic tectum of the Xenopus tadpole.**

AS Hamodi, KG Pratt. From the Department of Zoology & Physiology, University of Wyoming, Laramie, WY.

The caudal edge of the optic tectum of the Xenopus tadpole consists of a proliferative zone where neural progenitor cells give rise to neurons. These immature neurons then migrate rostrally into the tectum proper where they are incorporated into the retinotectal circuit (retinal ganglion cell axons projecting directly onto tectal neurons of the optic tectum). Ongoing incorporation of immature neurons from the caudal proliferative zone into the functional retinotectal circuit creates a spatial developmental gradient in which the most immature neurons are situated in the caudal portion of the optic tectum and the most mature in the rostral portion. Many aspects of neuronal maturation have been described along this rostrocaudal (RC) axis including increasing dendritic arbor complexity, increasing frequency and amplitudes of spontaneous synaptic events, increasing AMPA:NMDA ratios, and decreasing levels of calcium-permeable AMPA receptors. Much less has been reported about how the intrinsic properties may be changing along this RC axis. Since the way a neural circuit functions is determined by both a neuron's synaptic and intrinsic properties, and since there is much evidence that these two properties are functionally coordinated, we wondered how intrinsic properties may also be changing along the same axis. Therefore, using stage 48 tadpoles, we recorded in whole cell voltage clamp configuration from neurons residing in different parts of the tectum. So far, we identified a gradual and significant increase in both peak sodium and potassium currents along the RC axis. No significant difference in cell capacitance or input resistance was observed. Furthermore, we noticed that this location-dependent increase in intrinsic currents is correlated
with an increase in the amount of synaptic input received by individual neurons. This suggests that the development of intrinsic currents could be an activity-dependent process. We begin to test this possibility by altering the amount or pattern of synaptic activity received by the tectal neurons. Preliminary data suggest that chronic visual deprivation may disrupt the normal maturational gradient of intrinsic properties.

EXPERTISE: Electrophysiology

12) **The role of the neurokinin 3 receptor in neurite outgrowth.**

CJ Hoekstra, EW Kinney-Lang, FW Flynn. From the Department of Neuroscience, University of Wyoming, Laramie, WY.

Actin filaments are the helical polymers of the actin protein. Although dispersed throughout the cell, they are most highly concentrated in the cell cortex. Several cell cortical structures such as filopodia and lamellipodia are known to provide independent movement to a cell through lamellipodial motors. Another way in which actin filaments work within the cell is by promoting spiky bundles which protrude from the cell body and sense chemical cues in the environment. These cues encourage the cell to move, but may play a more important factor in determining cell morphology and filipodial outgrowth, particularly in the human nervous system. In the beginning of life, neurons must be guided by molecular and chemical cues in order to reach their correct targets in the brain. These chemicals are sensed by microspike filopodia on the extending tip of the growth cone. Once filopodia sense guidance chemicals they polymerize and motors allow for actin reorganization. Reorganization gives cells the ability to grow further projections that will become axons, dendrites, and dendritic spines. Because these processes are indistinguishable early in their development, they are given the term neurites. During development, many chemicals that function as neurotransmitters in the adult appear to have a dual role in promoting neurite outgrowth in the embryonic brain. GABA, Dopamine, Serotonin, and Substance P all appear to have neurotrophic effects on immature neurons. Another possible dual-acting neurotransmitter is the Neurokinin B (NKB) ligand which activates the Neurokinin 3 Receptor (NK3R). NK3R appears early in development (E12), and its early appearance raises the question if NK3R plays a role in early brain wiring. The NK3R is a g-protein coupled receptor (GPCR), and activation of NK3R recruits the 2,3 inositol triphosphate signaling cascade and promotes release of intracellular calcium. Intracellular calcium affects neurite outgrowth through the stabilization of actin filaments, and therefore may mediate the effects of NK3R-induced neurite outgrowth. Agonists for the NK3R are presently in clinical testing for the treatment of schizophrenia, which is categorized by miswiring of the developing brain. Understanding mechanisms underlying pathogenic brain development is of paramount importance to understanding and treating neuropsychiatric diseases.

EXPERTISE: Confocal Microscopy, Live Cell Imaging, Immunohistochemistry, Cell Transfections, Maintenence of Cell Lines, Plasmid Extraction, Mathmatical Modeling, Reverse Transcriptase PCR, Nuclear Isolation, Transmission Electron Microscopy.

13) **Connexin 35b in zebrafish spinal cord development.**

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

This project explores the role of connexin 35b (Cx35b) in zebrafish spinal cord development by defining its expression pattern in the developing spinal cord as well as defects that arise upon Cx35b loss or knockdown. In other vertebrates, Cx35b orthologues show predominantly neuronal expression. We find that cx35b expression begins during early embryonic stages, with expression detectable by RT-PCR at 50% epiboly, which is during neural induction but before genesis of post-mitotic neurons. From our whole-mount fluorescent RNA in situ hybridization studies, we find cx35b transcripts in the spinal cord by 17 hours post-fertilization (hpf). Additionally, these transcripts are preferentially localized to the ventral spinal cord. Our preliminary results using fluorescent RNA in situ hybridization in transgenic lines as well as transient expression in a BAC transgenic support cx35b expression in ventrally projecting secondary motor neurons (SMNs), dorsally projecting SMNs, dorsal-ventral SMNs, ventral descending (VeLD) inhibitory interneurons, circumferential descending (CiD) excitatory premotor interneurons, and unipolar commissural descending (UCoD) excitatory interneurons. The results of preliminary Cx35b knockdown/knockout suggest a requirement in motor axon pathfinding. Since motor axon pathfinding has been shown to be a process regulated by activity in other models, we are testing the possibility Cx35b may modulate activity patterns and thereby regulate motor neuron axon trajectories. We are using a combination of molecular, pharmacologic, genetic, and imaging techniques to determine whether Cx35b regulates spontaneous activity in the developing spinal cord. In a first series of experiments, we have used a transgenically expressed calcium indicator (GCaMP-HS) to visualize spontaneous activity patterns over many developmental time periods in the presence and absence of Cx35b expression. The results of these studies will provide insights into developmental mechanisms that require Cx35b.

EXPERTISE: IHC, ISH, calcium imaging and analysis

14) **Embryonic GABAB receptor blockade alters cell migration, adult hypothalamic structure, HPA axis activation, and anxiety- and depression-like behaviors in mice.**

C Nash[1], M Steigerwald[1], M Stratton[1,2], T Budefeld[3], D Carbone[4], R Handa[4], G Majdic[3], S Tobet[1,5]. From the [1]Department of Biomedical Sciences and [2]Molecular, Cellular and Integrative Neurosciences Program at Colorado State University, Fort Collins, CO; [3]Center for Animal Genomics, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia, SI-1000; [4]Department of Basic Medical Sciences, University of Arizona College of Medicine, Phoenix, AZ; [5]School of Biomedical Engineering, Colorado State University, Fort Collins, CO.

Neurons of the paraventricular nucleus of the hypothalamus (PVN) regulate the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. Previous experiments have shown that mice lacking functional GABAB receptors have sex specific alterations in PVN cell placement and protein expression and altered anxiety-like and depression-like behaviors. The current work tested whether alterations in GABAB receptor signaling that influence cell movements in the developing PVN also might cause lifelong changes in PVN cytoarchitecture and physiology and behaviors related to HPA axis function. Maternal treatment with a GABAB receptor antagonist CGP 55845 resulted in offspring with female-selective alterations in the placement of cells containing estrogen receptor α in the region of the PVN and anxiety-like behavior (elevate plus maze) in adulthood. Males and females displayed decreased depression-like behaviors (tail suspension and sucrose preference), which correlated with decreased HPA axis activation (immunoreactive FOS in PVN and plasma corticosterone). This work highlights the importance of GABAB signaling for PVN development and the expression of multiple complex behaviors in adulthood. The replication of cytoarchitectural and behavioral phenotypes of GABAB receptor knockout mice by antagonist treatment during a critical period of development emphasizes the role of GABA in a key fetal antecedent mechanism.

15) **Role of the prion protein in olfactory system development.**

LE Parrie, RA Bessen.. From the Prion Research Center, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO.

The cellular prion protein (PrPC) is an endogenously expressed, cell-membrane associated protein, most well-known for its role in misfolded prion protein diseases such as Creutzfeld-Jacob and Chronic wasting disease. Although PrPC has been associated with diverse processes including cell signaling, neurogenesis and neuroprotection, its exact physiological function remains ambiguous. The goal of this study is to determine the role of PrPC in adult neurogenesis, using the murine olfactory system model. Neurons within the olfactory system are capable of undergoing regeneration and integration even into adulthood, making it an ideal model for this study. Additionally, recent studies have used olfactory sensory neuron (OSN) precursors for cell-based treatment in a Parkinson's disease model. While this is a promising technique that may translate to other neurodegenerative diseases, including prion diseases, we must first determine the role of PrPC in the development of OSN precursors. Here we assay features of neurodevelopment utilizing immunohistochemistry (IHC), quantitative real-time PCR (qPCR), and confocal microscopy techniques. We will examine proliferation, differentiation, targeting, and migration of the OSN lineage in wildtype, PrP-overexpressing, and PrP-null animals. To begin investigating the role of PrPC in proliferation, adult wildtype and PrP-null mice were treated with BrdU and tissues were collected at 24 hrs, 1 week, and 2 weeks after BrdU injection. Results to date suggest that PrPC is involved in regulating olfactory cell proliferation, whereby an absence of endogenous prion results in an increased cell number in the septal olfactory sensory epithelium (OSE). This data indicates a putative role for PrPC in adult OSE neurogenesis.

16) **Protocadherin10a acts downstream of prdm1a in The formation of neural crest-derived pigment cells.**

CC Rossi, J Williams, L Hernandez, KB Artinger. From the Department of Craniofacial Biology, University of Colorado Anschutz Medical Campus, Aurora CO.

Protocadherins are genes that share an extracellular domain with classical cadherins but vary with respect to their cytoplasmic domains and functions. protocadherin10a (pcdh10a) has been implicated in several types of cancer in humans and is expressed in the nervous system, but its role during development is largely unknown. Because the transcription factor prdm1a plays a key role in the specification of embryonic neural crest cells (NCCs), we examined the expression profile of multiple genes during key stages of NCC development in zebrafish embryos using microarray analysis. We determined that pcdh10a expression is down-regulated 1.3 fold in 25 hour post-fertilization zebrafish embryos mutant for the prdm1a ($p = 0.02$). pcdh10a is expressed in a subset of late-migrating NCCs and its expression in migratory NCCs is absent in prdm1a mutant embryos as evidenced by in situ hybridization. In embryos injected with a Morpholino against pcdh10a, NCCs are specified

normally, but a decrease in the number of neural crest-derived pigment cells is observed, suggesting that the subset of migrating NCCs that express pcdh10a contributes to the pigment cell lineage. Embryos deficient in prdm1a have previously been shown to display reduced pigment cells, and we show here that injection of pcdh10a mRNA partially rescues this phenotype. pcdh10a expression in migrating NCCs is also decreased in embryos with a mutation in tfap2a, another zebrafish mutant in which some NCCs, including pigment cells, fail to develop normally. We conclude that pcdh10a is important in the development of neural crest-derived pigment cells and acts downstream of prdm1a (and potentially tfap2a) in the development of these cells.

17) **Characterization of neonatal seizures in an animal model of hypoxic-ischemic encephalopathy.**
D Sampath, A White, Y Raol. From the Department of Paediatric Neurology, School of Medicine, Anschutz Medical Campus, University of Colorado Denver, CO.
Rationale: Hypoxic-ischemic encephalopathy (HIE) is one of the most common causes of seizures in full-term infants. Hypoxia–Ischemia (HI) induced injury in neonatal rats is a commonly used model to study acute as well as chronic changes caused by HIE. In the current study, we characterize HI-induced acute neonatal seizures and changes in the EEG of an animal model of HIE.
Method: To record EEG, rat pups were implanted with subdural electrodes on postnatal day 6 (P6). To induce HI, the right carotid artery of P7 pup was ligated and then exposed to hypoxia for 2 hours. Electroclinical seizures were defined as an EEG pattern that differed from background in either amplitude or frequency or both, evolved
over time, contained spikes or sharps, lasting for 10 seconds or more, and were associated with a change in the rat's behavior. Electrographic seizures were defined as a seizure observed on the EEG but were not associated with changes in behavior of the rat.
Results: Analysis of video-EEG recordings revealed that the rat pups exhibit multiple electroclinical seizures (15.80 ± 9.47; mean ± SD; n = 5) during 2 hours of hypoxia. The first electroclinical seizure was observed 11.56 + 20.46 minutes (mean ± SD; n=5) after initiation of hypoxia. The total duration of electroclinical seizures during 2 hours of hypoxia was 5.93 + 2.97 minutes (mean ± SD; n = 5). An electrographic seizure was observed in one rat during hypoxia (n = 5). HI rat pups have multiple acute electroclinical seizures in the first 2 hours after hypoxia (17.20 ± 20.29; mean ± SD; n = 5). The rat pups continued to show electroclinical seizures even 24 hours after HI (23.80 ± 34.01 episodes/4 hour VEEG monitoring; n = 5). Behavioral seizures included forelimb and hind limb clonus, vibratory tonic seizures, whole body jerks, forelimb jerks, facial automatisms, tail stiffening, forelimb and hind limb paddling, thrashing and circling.
Conclusion: In this model of neonatal HI, we found that rats have multiple seizures during and immediately after hypoxia. The seizures were persistent even 24 hours after HI.
Acknowledgment: Supported by funding from the CURE (YHR), K08 NS053610-05 (AW) and NICHD RO1HD065534 (YHR).
EXPERTISE: EEG, Generating hypoxic-Ischemic Injury in neonatal rat pups brain.

18) **FMRP- and miRNA-mediated regulation of synapse structure in drosophila.**
BA Symmes, L Rozeboom, SA Barbee. From the Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver CO, USA.
The Fragile-X Mental Retardation Protein (FMRP) is a regulator of synaptic structure and function. FMRP interacts with components of the microRNA (miRNA) pathway and with specific miRNAs involved in controlling synapse structure. Whether FMRP and miRNAs are mutually dependent to regulate this process is unknown. In this study, we present recent data for two approaches we are taking to address this question. First, we investigate the translational control of Futsch—Drosophila homolog of the mammalian microtuble-associated protein (MAP1B)—a known target of translational repression by FMRP. Using an in vitro dual-luciferase assay, we show that four neuronal miRNAs (miR-9a/b/c and miR-315) predicted by in silico analysis to bind to Futsch, can specifically repress translation of a Futsch 3'UTR reporter. However, knock-down of endogenous levels of these miRNAs does not result in a corresponding increase in Futsch reporter levels. Interestingly, using a similar assay, we find that FMRP does not regulate expression of the Futsch reporter via the 3'UTR or 5'UTR. Moreover, immunoprecipitation of FMRP-containing RNP (ribonucleoprotein) complexes indicates there is no physical interaction between these miRNAs and FMRP. Together, these data suggest that FMRP and these miRNAs may regulate Futsch translation independently.Second, we take a broader approach to identify all miRNAs that interact with FMRP. We combine large-scale immunopurification of FMRP-containing complexes with next generation sequencing technologies (RNA-seq) in an unbiased approach to identify all small RNAs (sRNAs) and mRNAs associated in RNPs with FMRP. We will then use in silico analysis to identify candidate

miRNA/mRNA combinations that may play a role in the control of synapse structure. Current progress using this approach will be presented.
EXPERTISE: qRT-PCR, celll culture, protein gel, Western Blot, immunohistochemistry, Gateway cloning, Drosophila genetic crosses, dual-luciferase assay, immunoprecipitation

19) **Lipid microdomains regulate neuronal endocytic mechanisms during development.**
X Tang[2], P Baskaran[2], K Hognason[2], J Potian[3], J McArdle[3],B Thyagarajan[1,2,*]. From the [1]Program in Neuroscience and [2]School of Pharmacy at the University of Wyoming, Laramie, WY; [3]New Jersey Medical School at the University of Massachusetts-Dartmouth, Newark, NJ. *Corresponding author.
Phosphatidyl inositol (4,5) bisphosphate (PIP2) rich lipid microdomains (LMD) serve as scaffolds for the interaction of several signaling molecules. Many neurotoxins and pathogens use this scaffold for their endocytic processes. Here we demonstrate that neonatal mice less than 7 days (< P7) of age are resistant to the inhibitory effects of BoNT/A and that cholesterol depletion by methyl-β-cyclodextrin (MβCD; depletes membrane cholesterol) sensitized these mice to BoNT/A. In vitro, acute exposure to 10 pM BoNT/A for 90 min severely inhibited nerve-evoked muscle twitches and endplate potentials (EPPs) for phrenic-diaphragm nerve-muscle preparations (NMP) isolated from adult mice. In contrast, twitches and EPPs of NMP isolated from < P7 mice were not affected by BoNT/A. Remarkably, when < P7 mice were injected with MβCD (3 µl of 10 mM) prior to BoNT/A exposure, their TSR was inhibited within 24 hrs. Similarly, twitch tension and amplitude of EPPs recorded from NMP isolated from < P7 mice were dramatically reduced when exposed to 10 pM BoNT/A following a 30 min pretreatment with 10 mM MβCD. In vitro studies demonstrated that control NMP of < P7 mice did not endocytose BoNT/A while MβCD pretreatment significantly increased BoNT/A endocytosis. Experiments addressed to determine the mechanism underlying this revealed that neonatal NMJ expressed higher levels of synaptojanin-1 (SYNJ-1; presynaptic PIP2 phosphatase) and lower levels of SV2 (BoNT/A receptor) and that MβCD pretreatment (10 mM; 1 hr at 22 °C) altered the subcellular localization of SYNJ-1 and also increased the interaction between SV2 and BoNT/A. MβCD alone or αCD (does not deplete cholesterol) did not affect the TSR, muscle twitch tension, or ACh release of neonatal NMP. Our data demonstrate the critical role of cholesterol rich lipid microdomains in the regulation of endocytic mechanisms during development. Acknowledgment: 1001070G NIH/DHHS COBRE (B Thyagarajan).
EXPERTISE: Cell culture, immunocytochemistry, PCR and Immunoblotting

Disorders of the Nervous System

20) **Neural stem cells and hydrogels: enhancing neural tissue engineering potential.**
ER Aurand, J Wagner, C Lanning, KB Bjugstad. From the Neuroscience Program, Department of Bioengineering, and Department of Pediatrics, University of Colorado - Anschutz Medical Campus, Aurora, CO.
Hydrogels used for neural tissue engineering must mimic the properties of brain tissue in addition to being customizable and therapeutically functional. Currently, hydrogels used for neural tissue engineering are not thoroughly characterized for their chemical, physical, and mechanical properties. The goal of this research was to create a well-characterized hydrogel using materials suitable for the implantation of neural cells. Hydrogels were created from a range of ratios of hyaluronic acid (HA) to poly(ethylene glycol) (PEG). Hydrogels were assessed for the physical properties of polymerization and degradation, the mechanical property of compressive modulus, and the cytocompatibility with encapsulated fetal neural precursor cells (fNPC). The polymerization and degradation rates of the HA:PEG hydrogels were a function of the amount of HA incorporated into the hydrogel. The compressive modulus (hydrogel stiffness) was also a function of the HA content and was found to increase with increasing HA content. In addition to physical and mechanical characterization, fNPC were encapsulated within a three-dimensional (3D) HA:PEG hydrogel environment to establish biocompatibility. Results demonstrated that 3D cultures had good survival/proliferation, with an average survival of 122.5% compared to only 34.6% in TCP controls after 24 hours. fNPC survival was dependent on the relative proportion of HA or PEG. At low concentrations of PEG, fNPC survival was high, regardless of HA content. At high concentrations of PEG, fNPC survival was dependent on the amount of HA. Three-week fNPC differentiation was also addressed, and was shown to be influenced by hydrogel mechanical properties. Generally, greater numbers of astrocytes were observed on stiffer hydrogels, while increased numbers of neurons were seen on softer hydrogels. These results indicate that there can be a wide range of HA:PEG-based hydrogels which are compatible with neural cells and that cell fate can be altered based on hydrogel properties.

21) **Augmentation of mitochondrial glutathione transport renders a motor neuron cell line resistant to oxidative stress.**

S Brock[1], H Wilkins[1], DA Linseman[1,2]. From the[1]Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver, CO; [2]Research Service, Veterans Affairs Medical Center, Denver, CO.

Oxidative stress, particularly at the level of mitochondria, is a significant factor in the pathogenesis of neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS). Mitochondria generate free radicals as a byprduct of electron transport and therefore, contain essential free radical scavenging systems such as the antioxidant glutathione (GSH). GSH is synthesized solely in the cytoplasm and has a net negative charge so it cannot simply diffuse across the inner membrane of the mitochondria without active facilitation. Mitochondrial GSH is a key endogenous antioxidant and maintenance of the mitochondrial GSH pool is critical for cell survival. Previous studies have demonstrated that an inner mitochondrial membrane anion transporter, the 2-oxoglutarate carrier (OGC), is capable of transporting GSH into mitochondria within hepatic and renal cells. Nothing is known about the role of mitochondrial GSH transport in protecting motor neurons from oxidative damage such as that observed in ALS. Here, we stably overexpressed OGC in the NSC34 mouse motor neuron-like cell line. Two stable NSC34 cell lines consistently demonstrated overexpression of OGC. Each of these cell lines displayed a significant and specific increase in mitochondrial GSH content compared to parental NSC34 cells. Both OGC overexpressing cell lines exhibited marked resistance to hydrogen peroxide induced cell death. Preincubation with phenylsuccinate; an OGC inhibitor, resensitized both of the OGC overexpressing cell lines to oxidative stress and reduced levels of mitochondrial GSH. Finally, the OGC overexpressing cell lines displayed significant upregulation of Bcl-2 expression. These data suggest that augmentation of mitochondrial GSH transport by overexpression of OGC renders NSC34 cells resistant to conditions of increased oxidative stress. Moreover, increases in mitochondrial GSH significantly increase the expression of Bcl-2, a protein which we have previously shown to interact with OGC in a GSH-depenent manner. These findings suggest that modulation of mitochondrial GSH transport could be a novel therapeutic approach for neurodegenerative diseases like ALS.

EXPERTISE: Western blotting, tissue culture

22) **Dietary docosahexaenoic acid (DHA) improves behavioral and biomarker outcomes when provided before or after experimental diffuse brain injury.**

CM Butt, J Lifshitz, J Jones, N Salem, Jr., JR Pauly. From Neuroscience, DSM Nutritional Products, Boulder, CO; the Spinal Cord and Brain Injury Research Center, University of Kentucky, Lexington, KY.

Clinical data indicate that DHA is important for brain development (Carlson [2009] Am J Clin Nutr 89:678S) and protection from age-related cognitive decline (Yurko-Mauro et al. [2010] Alzheimers Dement 6:456). The pleiotropic effects of DHA include activation of anti-apoptotic genes, decreased inflammatory signals and decreased oxidative stress (Su [2010] J Nutr Biochem 21:364). Recent experiments have suggested that DHA protects against brain damage when provided orally before weight-drop injury (Mills et al. [2011] Neurosurgery 68:475) or after lateral fluid percussion injury (Wu et al. [2011] J Neurotrauma 28:2113). However, it is unknown whether agreement exists between these prophylactic and therapeutic effects of DHA in the same brain injury model and when using the same commercially available source of DHA. We sought to provide consensus on this issue by formulating DHA-containing diets (1% of total fatty acids) with DHASCO® oil, feeding the DHA diets to male, Sprague-Dawley rats before or after midline fluid percussion injury (mFPI), and comparing the effects of dietary DHA to those of a DHA-free diet on spatial task performance (Morris water maze), sensory sensitivity (whisker nuisance task), and microglial activation ([3H]PK11195 autoradiography) after brain injury. Animals receiving DHA for 4 weeks before or for 15-22 days after mFPI exhibited significant improvement on the learning task (days 15-18 post-injury) and significant reductions in sensory sensitivity (day 22 post-injury) than animals maintained on the DHA-free diet. However, in comparison to animals on the DHA-free diet, significant decreases in microglial activation were only observed in animals given DHA after the injury. These findings suggest that the protective mechanisms of DHA prophylaxis differ in some respects from those observed during the therapeutic period. Furthermore, converging data support DHA supplementation as a means to decrease the effects of brain injury. Future experiments should address whether dietary DHA provided before and after brain injury confers any additional benefits.

EXPERTISE: immunohistochemistry, autoradiography, tissue culture, behavioral testing

23) **c-Myb and its possible role in schizophrenia.**
CJ Cabrera Montalvo, JA Stitzel. From the Integrative Physiology Department, Institute of Behavioral Genetics at the University of Colorado-Boulder, Boulder, CO.
Previous research has shown that knocking out c-myb, a transcription factor, in the mouse brain causes hypertrophy of the olfactory bulbs, enlarged ventricles, ependymal cell anomalies and a decrease in neurogenesis. These changes are similar to the anatomical anomalies seen in subjects with schizophrenia. We have therefore characterized by in-situ hybridization where c-myb is expressed in the adult brain and partly in the embryonic brain if 15 days, and notice it seems to be expressed in areas affected in Schizophrenia. To further examine the effects of c-myb in the brain, we wanted to see if any genes that were disrupted in schizophrenia were also disrupted by the presence of this transcription factor. We therefore identified by luciferase assays fragments of the promoter of CHRNA7, downregulated in schzophrenia, and saw which fragments containing the consensus sequence for c-Myb caused a change in abundance of the protein compared to a control plasmid. The fragments that caused a change in abundance were further studied by Electrophoretic Mobility Shift Assays (EMSA), and it was found that one indeed is a binding site for c-Myb. We also looked at gene regulation of Chrna7 and other genes that are disrupted in schizophrenia like Disc1, Sox10 and VegfA. Preliminary qRT PCR data from SH-SY5Y cell culture the suggests c-Myb may be affecting, whether directly or indirectly, the concentrations of CHRNA7, and qRT PCR data from a mouse c-Myb knockdown model correlates it with levels of CHRNA7, Disc1, Sox10 and Vegf-A. Future studies include corroborating the data with other controls, determining the location of c-Myb in other stages of the embryonic brain, and evaluating these effects in other cell lines.
EXPERTISE: Real time qPCR, Electrophoretic Shift Mobility Assay

24) **Early-life iron intake modulates late-life outcomes in Huntington's disease mice.**
J Chen[1], J Fox[1], J Moline[2], J Duce[3], I Volitakis[3], A Bush[3]. From the [1]Department of Veterinary Sciences and Interdepartment Neuroscience Program, University of Wyoming; [2]Department of Veterinary Sciences, University of Wyoming; [3]University of Melbourne, Australia.
Huntington's disease (HD) is an autosomal dominant chronic progressive disorder caused by an expanded CAG repeat in the huntingtin gene which encodes a polyglutamine repeat in huntingtin protein. Iron accumulates in the central nervous system (CNS) in a number of neurodegenerative conditions including HD. However, how this elevation contributes to disease process is not fully understood. There is evidence showing that manipulation of brain iron alters progression of neurodegenerative diseases. Here we tested whether a nutritionally relevant elevation of iron intake in early life potentiates HD in R6/2 mice. Pups were dosed with saline vehicle or carbonyl iron daily from days 10 to 17. Spontaneous in-cage wheel activity measurements were taken at 5 and 10 weeks of age. Measurements of rota-rod were taken at 5, 8 and 12 weeks. Mice were sacrificed at 13 weeks at late-stage disease. Iron treated HD mice had less wheel activity at 10 weeks suggesting increased motor impairment. Brain biochemical studies showed iron treatment significantly increased levels of the oxidative stress and energetic markers, oxidized glutathione (GSSG) and lactate, in HD but not wild-type mice. Stereology studies showed that early-life elevated iron intake resulted in decreased neuronal cell body volume in striatum and cortex of HD mice but not wild-type mice, consistent with potentiation of neurodegeneration. However, brain regional iron levels were not significantly elevated in iron supplemented HD mice. Our findings indicate that elevated early life iron intake potentiates HD in R6/2 HD mice. Increased brain iron levels are not required for iron potentiation of HD. Findings are relevant to nutrition in human HD and possibly related neurodegenerative diseases. Supported by neuroscience center grant NIH P30 GM103398-02 and 1R01NS079450-01A1.
EXPERTISE: Behavior testing, stereology analysis

25) **Toll Like Receptor 4 (TLR4) antagonism suppresses cocaine reward.**
TA Cochran, AL Northcutt, EL Galer, ME Haas, MR Hutchinson[1], CE O'Neill, X Wang, NE Miles, J Amat, SF Maier, RK Bachtell, KC Rice[2], LR Watkins. From the Department of Psychology and Neuroscience at University of Colorado, Boulder; [1]Discipline of Physiology, University of Adelaide, Australia; [2]Chemical Biology Research Branch, National Institute on Drug Abuse, Rockville, Maryland.
Historically, the rewarding effects of drugs have been attributed to neuronal responses, particularly those of the mesolimbic dopamine pathway. Recent evidence suggests that brain immune responses contribute to drug reward; for instance, morphine can activate proinflammatory signaling through interactions with TLR4, an innate immune pattern recognition receptor expressed principally on glial cells in the CNS. We have shown that the non-opioid (+)-isomer of naloxone is a selective TLR4 antagonist and co-administration with morphine

blocks drug reward. We now have evidence that cocaine is also a TLR4 agonist and induces a proinflammatory response. The following series of studies were conducted to explore possible TLR4 involvement in cocaine reward. Conditioned place preference (CPP) was suppressed when (+)-naloxone was co-administered with cocaine. Additionally, (+)-naloxone blocked cocaine-induced increases of dopamine (DA) in the nucleus accumbens (NAc) shell. To further investigate whether cocaine could induce proinflammatory signaling in regions of the mesolimbic DA pathway, a timecourse RT-PCR study was conducted. Of particular interest, interleukin-1 beta (IL-1β) mRNA expression was up-regulated in the VTA, but not in the NAc or prefrontal cortex, and this up-regulation was attenuated with (+)-naloxone. Based on these results, we investigated the effects of intra-VTA TLR4 antagonism or IL-1β signaling blockade; in both cases, cocaine induced dopamine increases in the NAc shell were attenuated. Finally, in a self-administration paradigm, (+)-naltrexone dose-dependently suppressed reinstatement to cocaine seeking. Collectively, these studies indicate that the rewarding effects of cocaine are mediated by the activation of TLR4 receptors and subsequent proinflammatory signaling. Not only do these findings expand and redefine our understanding of mechanisms underlying cocaine reward, but also indicate that TLR4 may be a promising target for pharmacological intervention to treat cocaine addiction.

26) SK Grotewold, V Wall, C Hayter, A Bowman, D Goodell, ST Bland. From the Department of Psychology, University of Colorado-Denver, Denver, CO.

Adverse experiences during the critical period of adolescence can produce either vulnerability or resilience to challenges later in life. Isolation rearing, a model of adolescent adversity in rats and other social species, consists of housing animals in isolation throughout the adolescent period. Isolation rearing has been previously shown to produce alterations in social behavior and responses to rewarding drugs. Here we explored the effects of isolation rearing on the responses of dopamine and serotonin in the nucleus accumbens (NAcc) produced by exposure to cocaine or a social cue (a novel same-sex juvenile rat) or to the combination of cocaine and a social cue. Male and female Sprague-Dawley rats were housed in either same-sex groups of 3 (Group) or individually housed (ISO) for 4 weeks beginning at postnatal day 22 (P22). At P50 cannulae were implanted in the NAcc shell and after 1 week of recovery in vivo microdialysis was performed. After a 1 hour baseline period, rats received either 2 mg/kg cocaine or equivolume saline. Half of each drug group were then exposed to a social cue for 10 minutes. Samples were assessed with HPLC. A social cue alone had no effect on NAcc dopamine in either Group or ISO rats. Cocaine produced an increase in NAcc dopamine levels in Group rats both in the presence and the absence of a social cue. In contrast, in ISO rats cocaine produced a blunted dopamine response, and only the combination of cocaine and social cue produced an increase in dopamine levels. A similar pattern of results was observed for serotonin, but this did not reach significance. These results indicate a blunted monoamine response to cocaine after isolation rearing, but suggest that cocaine can synergize with a social cue in ISO rats to potentiate NAcc monoamines, especially dopamine.

27) **Toll Like Receptor 4 antagonism attenuates cocaine and methamphetamine induced dopamine increases in the nucleus accumbens**

ME Haas[1], AL Northcutt[1], EL Galer[1], TA Cochran[1], MR Hutchinson[2], X. Wang[1], NE Mile[s1], SF Maier[1], KC Rice[3], LR Watkins[1]. From the [1]Department of Psychology & Neuroscience, University of Colorado, Boulder; [2]Discipline of Physiology, University of Adelaide, Australia; [3]Chemical Biology Research Branch, National Institute on Drug Abuse, Maryland.

Cocaine, a psychostimulant drug, is historically believed to exert its rewarding effects by blocking the dopamine transporter (DAT) causing an accumulation of dopamine (DA) in the nucleus accumbens (NAc). However, findings are contradictory as to whether the blockade of DAT alone is sufficient to induce rewarding effects. Additionally, it has been shown that rodents will self administer cocaine into the ventral tegmental area (VTA). These findings suggest that there may be another site of action for cocaine. In silico modeling indicates that cocaine interacts with TLR4, which has been corroborated with further biophysical characterization of cocaine-TLR4 interactions. In-vivo microdialysis data demonstrate that (+)-naloxone antagonism of TLR4 blocks cocaine-induced increases of NAc DA. PCR data show that cocaine induces up-regulation of mRNA for proinflammatory markers in regions of the brain relevant to drug reward, including the VTA and NAc. Methamphetamine (MA), another psychostimulant drug, is also believed to exert its effects by binding to and blocking the DAT. However unlike cocaine, MA reverses the DAT causing excess DA to accumulate in the NAc shell. In silico modeling shows that MA docks at the TLR4-MD2 complex, much like cocaine. However in-vivo microdialysis findings reveal that a 3-fold increase of (+)-naloxone dosing is required to attenuate MA-induced NAc DA increases. PCR data show that MA elicits a proinflammatory response in the VTA and the NAc. These

findings indicate that TLR4 signaling is critical for the effects of cocaine in the mesolimbic dopamine pathway and could be a promising target for the development of pharmacological interventions for cocaine addiction. While TLR4 might play a role in MA reward, our findings suggest that this interaction may not be as critical as cocaine, as indicated not only by the 3-fold (+)-naloxone increase, but by further biophysical characterization, indicating that MA has a lower affinity for the TLR4 complex than cocaine.

28) **Selenium supplementation is neuroprotective in mouse Huntington's disease and normalizes liver selenium.**

Z Lu[*1,2], J Chen[*1,2], E Marks[*1,2], J Moline[1], L Barrows[1], M Raisbeck[1], JH Fox[1,2]. *equal contribution. From the [1]Department of Veterinary Sciences; the [2]Neuroscience Graduate Program, University of Wyoming, Laramie, WY.

Huntington's disease (HD) is a progressive neurologic disorder caused by polyglutamine-expanded mutant huntingtin protein (mhtt) which misfolds and accumulates in neurons resulting in selective brain degeneration. Selenium is an essential nutrient that is required for normal brain function. Defects of brain selenium metabolism can induce neurodegeneration. We tested the effect of selenium supplementation in N171-82Q Huntington's disease mice. Female mhtt (+/-) and mhtt (-/-) mice were provided with two concentrations of selenite in drinking water and / or control water, from 6-14 weeks of age. Mice were examined for behavioral effects as well as brain biochemical and structural changes at 14 weeks corresponding to late-stage disease. Selenium supplementation of N171-82Q mice: (1) increased motor endurance, (2) protected against loss of brain mass, (3) increased striatal dopamine and cyclic-AMP-regulated phosphoprotein and (5) reversed elevations of oxidized glutathione concentration in frontal cortex. We are currently testing whether there is a systemic defect in selenium metabolism in HD mice. To this end we quantified liver selenium, the major site of selenium storage in the body, and found a significant reduction in HD mice compared to wild-type mice; this decrease was reversed in the selenium supplemented HD mice. In toto, our findings indicate that selenium supplementation provides some protection in HD mice. Further, while HD is a neurodegenerative disease our data supports the possibility of altered whole body selenium metabolism having a contributory role in the disease process. Sources of support: Funding was provided by University of Wyoming Neuroscience COBRE (5P20RR015640-10) and Hatch Project #WYO-438-09 (JF).

EXPERTISE: immunofluorescence microscopy, glutathione assay, PCR genotyping, stereology

29) **Does inhibition of the 5-LO leukotriene synthesis pathway with MK-886 ameliorate disruption of the BBB and edema following fluid percussion injury?**

P Šerbredzija, CE Corser-Jensen, DJ Goodell, RC Murphy, and KA Heidenreich. From the Department of Pharmacology and Neuroscience Program, University of Colorado, Anschutz Medical Campus, Aurora, CO.

Traumatic brain injury (TBI) is a non-degenerative, non-congenital insult to the brain from an external mechanical force leading to temporary or permanent impairments of cognitive and/or physical functions. TBI is a major cause of death and disability in all age groups. Major causes of TBI are falls, motor vehicle accidents, armed conflicts, and sports injuries. Primary injury from the initial impact site starts a chain of events called secondary injury which leads to additional morbidity and possibly death. Secondary injury includes but is not limited to disruption of the blood-brain barrier (BBB), which precedes edema and often progresses to a lethal increase in intracranial pressure. Although it is well understood that early intervention is necessary to prevent or ameliorate BBB disruption and edema following TBI, there is currently no effective treatment. Using a fluid percussion injury (FPI) model of TBI in rats, our lab previously found that TBI results in activation of a transcellular 5-lipooxygenase (5-LO) pathway that transiently increases the production of pro-inflammatory cysteinyl leukotrienes (LTs) in brain. We hypothesize that these LTs contribute to secondary brain injury, including disruption of the BBB and subsequent edema. To test the hypothesis, we used MK-886, an inhibitor of a key co-enzyme for LT biosynthesis called FLAP. Using magnetic resonance imaging (MRI), T1 post-gadolinium images showed significant BBB disruption in the ipsilateral leptomeninges from mechanical impact. This injury was not attenuated by MK-886. Swelling of the ipsilateral hemisphere observed in T2 images was significantly attenuated by MK-886. To measure BBB disruption in brain parenchyma, rats were injected with Evans blue (EB) and brain slices were imaged using fluorescence microscopy. Large numbers of EB-positive cells were detected in caudal and ventral regions of both cortex and hippocampus. Quantitation of EB-positive cells as well as the effect of MK-886 on EB extravasation is currently in progress. If FLAP inhibitors prove to block BBB disruption and edema in our animal model of TBI, translation to human clinical trials may be feasible.

EXPERTISE: Western blotting, immunohistochemistry, immunocytochemistry, cell culture, water maze/behavior testing

30) **Dysregulation of Rho or Rac elicits death of NSC34 cells and activation of these GTPases is altered in G93A mSOD1 mice.**

TR Stankiewicz, RJ Bouchard, DA Linseman. From the Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver, CO; Research Service, Veterans Affairs Medical Center, Denver, CO.

Several studies have demon-strated a critical function for Rho GTPase family members (i.e., Rac, Rho, Cdc42) in neuronal development and survival. Although a pro-survival function for Rac has been reported in several neuronal cell types, the antagonistic relationship be-tween Rac and Rho/ROCK signaling in neuronal survival remains poorly understood. In the current study, we examined the effects of a constitutive activator of Rho, CN03, on neuronal survival in vitro. CN03 potently and selectively activates Rho GTPase by deamidating glutamine 63 within the switch 2 region of the protein, essentially blocking GTPase activity and locking Rho in an active conformation. Our data demonstrate that treatment of NSC34 motor neuronal cells with CN03 results in significant cell death. We also examined the effects of targeted inhibition of Rac GTPase on neuronal survival. NSC23766 inhibits the activation of Rac by disrupting its interaction with the Rac-specific guanine nucleotide exchange factors (GEFs), Tiam1 and Trio. In a manner similar to constitutive activation of Rho, we demonstrate that treatment with NSC23766 induces cell death reminiscent of anoikis in NSC34 motor neuronal cells. These data suggest that the balance between Rac and Rho signaling is critical for motor neuronal survival. Moreover, in the G93A mutant Cu,Zn-super-oxide dis-mutase (SOD1) mouse model of amyotrophic lateral sclerosis (ALS), active Rac1-GTP immunoreactivity is markedly decreased in choline acetyltransferase (ChAT)-positive motor neurons of the lumbar spinal cord of end-stage mice when compared to age-matched wild type littermates. In addition, although immunoreactivity for total RhoB localizes to nuclei of ChAT-positive motor neu-rons from wild type mice, RhoB appears to mislocalize to motor neuronal pro-cesses in end-stage mice harboring the G93A mSOD1 mutation. Collectively, our data demonstrate that Rac and Rho are critical regulators of neuronal survival and as a result, disruptions in the balance of their activities may contribute to the etiology of motor neurodegenerative diseases such as ALS.

EXPERTISE: immunohistochemistry, immunocytochemistry, western blotting, cell and tissue culture, PCR

31) **Procyanidin B2 protects neurons from oxidative, nitrosative, and excitotoxic stress.**

TC Sutcliffe, AN Winter, DA Linseman Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver, CO and Research Service, Veterans Affairs Medical Center, Denver, CO.

The aberrant generation of oxygen and nitrogen free radicals can cause severe damage to key cellular components, resulting in cell apoptosis. Similarly, excitotoxicity leads to protease activation and mitochondrial dysfunction, which subsequently causes cell death. Each of these factors play critical roles in the neuronal cell death underlying neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS). Procyanidin B2 is a naturally occurring polyphenolic antioxidant compound that is found in high concentrations in cocoa, apples, and grapes. We examined the neuroprotective effects of procyanidin B2 in primary cultures of rat cerebellar granule neurons (CGNs) exposed to oxidative and nitrosative stressors, as well as to glutamate-induced excitotoxicity and non-depolarizing (5K) apoptotic medium. CGNs were preincubated for 6 hours with varying concentrations of procyanidin B2 and then neuronal stress was induced as described below. Oxidative stress was induced at the level of the mitochondria by treating CGNs with HA14-1, a compound that inhibits the function of the pro-survival Bcl-2 protein, and induced glutathione-sensitive apoptosis. Sodium nitroprusside (SNP), a nitric oxide generating compound, was used to induce nitrosative stress. Glutamate and glycine were used to induce excitotoxicity. We observed significant dose-dependent protection of CGNs with procyanidin B2 for all of the above stressors. We induced neuronal stress through the removal of depolarizing potassium and serum, a classical model of intrinsic apoptosis in CGNs. In contrast to the other insults, procyanidin B2 did not display significant protection against 5K-induced apoptosis at any concentration tested. Based on these findings, we hypothesize that procyanidin B2 offers neuronal protection principally as an antioxidant by scavenging reactive oxygen and nitrogen species instead of through modulating pro-survival or pro-apoptotic cell signaling pathways. These findings suggest that procyanidin B2 may be an effective neuroprotective agent for the treatment of neurodegenerative disorders like ALS, which involve oxidative, nitrosative, and excitotoxic damage to key neuronal populations.

32) **Neuroprotective efficacy and pharmacokinetics of novel para-phenyl substituted diindolylmethanes in a model of Parkinson's disease.**

B Trout, J Miller, R Hansen, S Safe, D Gustafson, R Tjalkens. From the Department of Environmental and Radiological Health Sciences and the College of Veterinary and Biomedical Sciences, Colorado State University, Fort Collins, CO.

There are no approved therapeutics that block the chronic inflammatory component of neurodegenerative diseases such as Parkinson's. This is partly because of poor distribution to the central nervous system for compounds with demonstrated efficacy in vitro. This study examined selected para-phenyl substituted diindolylmethane (C-DIM) compounds, which we previously demonstrated to be effective at decreasing glial-derived inflammatory gene expression in vitro. We postulated that the pharmacokinetic properties of C-DIM compounds would positively correlate with neuroprotective efficacy in a progressive model of Parkinson's disease (PD) in vivo. Pharmacokinetics and metabolism of 1,1-bis(3'-indolyl)-1-(p-methoxyphenyl)methane (C-DIM5), 1,1-bis(3'-indolyl)-1-(p-hydroxyphenyl)methane (C-DIM8), and 1,1-bis(3'-indolyl)-1-(p-chlorophenyl)methane (C-DIM12) were determined in plasma and brain of C57Bl/6 mice. Intravenous (1 mg/kg) and oral (10 mg/kg) doses were given to determine the optimal route of administration and putative metabolites were measured in plasma, liver, and urine. Oral dosage of C-DIM compounds displayed greater AUC, Cmax, and Tmax levels than intravenous administration. C-DIM12 exhibited distinguished pharmacokinetics of the selected C-DIMs, with an oral bioavailability of 42% in comparison of C-DIM8 (6%). Following pharmacokinetic studies, efficacy of C-DIM5, C-DIM8, and C-DIM12 (50 mg/kg, oral gavage) was established using a progressive, neuroinflammatory PD model employing MPTP and probenecid (MPTPp) over a period of 14 days. By first creating a lesion in the region of the brain affected in PD (substantia nigra) and then treating with anti-inflammatory C-DIMs, we determined that C-DIM5 and C-DIM12 demonstrated the greatest efficacy in attenuating the progressive loss of dopamine neurons.

EXPERTISE: Immunofluoresence, Stereology

33) **Mitochondrial glutathione transport is a key determinant of neuronal susceptibility to oxidative and nitrosative stress.**

HM Wilkens[1], D Kirchhof[1], E Manning[1], JW Joseph[2], DA Linseman[1,3,4]. From the [1]Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver, CO; [2]School of Pharmacy, University of Waterloo, Health Science Campus, Ontario, Canada; [3]Research Service, Veterans Affairs Medical Center, Denver, CO; [4]Division of Clinical Pharmacology and Toxicology, Department of Medicine and Neuroscience Program, University of Colorado Denver, Aurora, CO.

Mitochondrial oxidative stress significantly contributes to the underlying pathology of several devastating neurodegenerative disorders. Mitochondria are highly sensitive to the damaging effects of reactive oxygen and nitrogen species and therefore, these organelles are equipped with a number of free radical scavenging systems. In particular, the mitochondrial glutathione (GSH) pool is a critical antioxidant reserve that is derived entirely from the larger cytosolic pool via active transport. The mechanism of mitochondrial GSH transport has not been extensively studied in brain. However, the dicarboxylate (DIC) and 2-oxoglutarate (OGC) carriers localized to the inner mitochondrial membrane have been established as GSH transporters in liver and kidney. Here, we investigated the role of these carriers in protecting neurons from oxidative and nitrosative stress. Immunoblot analysis of DIC and OGC in primary cultures of rat cerebellar granule neurons (CGNs) and cerebellar astrocytes showed differential expression of these carriers, with CGNs expressing only DIC and astrocytes expressing both DIC and OGC. Consistent with these findings, butylmalonate specifically reduced mitochondrial GSH in CGNs, while both butylmalonate and phenylsuccinate diminished mitochondrial GSH in astrocytes. Moreover, pre-incubation with butylmalonate but not phenylsuccinate, significantly enhanced susceptibility of CGNs to oxidative and nitrosative stressors. This increased vulnerability was largely prevented by incubation with cell-permeable GSH monoethylester but not malate. Finally, knockdown of DIC with adenoviral-siRNA also rendered CGNs more susceptible to oxidative stress. These findings demonstrate that maintenance of the mitochondrial GSH pool via sustained mitochondrial GSH transport is essential to protect neurons from oxidative and nitrosative stress.

EXPERTISE: Immunocytochemistry, tissue culture, western blotting

34) **Chemical basis for the disparate neuroprotective effects of the anthocyanins, callistephin and kuromanin, against nitrosative stress.**

AN Winter, EK Ross, DA Linseman. From the Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver, CO

Oxidative and nitrosative stress have been implicated as major underlying causes of the neuronal cell death observed in a variety of neurodegenerative diseases. Antioxidant supplementation has provided promising insight into prospective treatment strategies for neurodegeneration; however, many antioxidants are protective against only the reactive oxygen species associated with oxidative stress, and cannot defend neurons from equally damaging reactive nitrogen species. In this study, we explore the capacity of two anthocyanins, callistephin and kuromanin, to protect cerebellar granule neurons (CGNs) from damage induced by either oxidative or nitrosative stress. While both compounds
protect CGNs equally under conditions of oxidative stress, a stark contrast is observed under conditions of nitrosative stress in which only kuromanin displays neuroprotection. This protection is mitigated by the over-expression of SOD1, suggesting a dependence upon superoxide. Based on these observations, we suggest that the presence of a catechol moiety on kuromanin lend it the unique ability to generate superoxide to act as a scavenger of nitric oxide, thereby preventing cellular apoptosis caused by nitrosative stress.

Neural Excitability, Synapse and Glia

35) **Structure and dynamics of the synaptophysin/VAMP2 complex.**

DJ Adams, CP Arthur, MHB Stowell. From the Department of Molecular Cellular and Developmental Biology at the University of Colorado, Boulder, CO.

Despite being one of the first identified and most abundant proteins in the chemical synapse, the function of Synaptophysin is still largely unknown. Synaptophysin has a well known interaction with the essential v-SNARE Synaptobrevin2 (VAMP2) and although the significance and function of this interaction has been debated, no conclusive data has yet been published. In order to learn more about the Synaptophysin/VAMP2 complex we determined the structure of the complex by single particle analysis and electron microscopy and have generated a model of the complex to compare to our published structure of the Synaptophysin homohexamer. We then used live cell imaging in primary neurons to determine if pharmacologic perturbations of the complex resulted in disruptions in neurotransmission. We found a striking decrease in the exocytosis kinetics upon stimulation of the neurons independent of the mechanism by which we disrupt the complex. These data constitute the first evidence that the Synaptophysin/VAMP2 complex plays an active role in synaptic transmission.

EXPERTISE: Primary Neuronal Cultures, Light Microscopy, Electron Microscopy, Image Analysis, Tissue Culture

36) **Uncovering roles for fractalkine in CD4+ T cell migration into the central nervous system in patients with relapsing remitting multiple sclerosis.**

K Blauth[1], X Zhang[1], K Marcus[1], D Bruce[1], M Chopra[1], L Troiani[1], S Markovic-Plese[1, 2]. From the [1]Department of Neurology; [2]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, NC.

Chemokines play roles in leukocyte trafficking into the CNS during the development of brain and spinal cord inflammatory lesions in patients with multiple sclerosis (MS). However, the role of fractalkine, a chemokine secreted by CNS neurons and endothelial cells, has not been extensively studied in the pathogenesis of the disease. Here we found that fractalkine levels are higher in both CSF and serum derived from RR MS patients in comparison to HCs, and that the fractalkine concentration is higher in CSF than in serum of both MS patients and HCs. We identified that fractalkine receptor (CX3CR1) and surface-bound intracellular adhesion molecule (ICAM)-1 are significantly upregulated on CD4+ T-cells derived from RR MS patients in comparison to CD4+ T-cells from HCs. Incubation of PBMCs in the presence of 1 ng/ml fractalkine upregulated cell surface expression of ICAM-1 on CD4+ T-cells derived from RR MS patients, but not on CD4+ T-cells from HCs. Fractalkine incubation increased IFN-γ secretion by CD4+ T cells isolated from MS patients, but not from HCs. CX3CR1 expression on CD4+ T cells derived from the CSF of MS patients compared to CD4+ T cells from the same patients' blood samples revealed higher levels of CX3CR1 on CD4+ T cells derived from the CSF than derived from the blood. Conclusion: This study suggests that the chemotactic effects of fractalkine, a chemokine secreted from the neurons, attracts CD4+ T cells into the CNS in vivo. Our results suggest that CD4+ T cell migration towards the CNS is mediated via fractalkine signaling through CX3CR1 and upregulation of ICAM-1, and is accompanied by an increase in IFN-γ production. Research supported by

NMSS grant # RG 4107A1/1.

37) **Arterial smooth muscle oxidant and calcium microdomain signaling.**
NL Chaplin, MM Frey, AM Fresquez, GC Amberg. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.
Calcium and redox-dependent biochemical processes are integral components of cellular biology. Concomitant disruption of calcium and redox homeostasis beyond physiological parameters is associated with many cardiovascular disorders including hypertension and stroke. The cardiovascular system appears to be particularly sensitive to perturbations in calcium and redox homeostasis. Accordingly, enhanced calcium entry and increased production of reactive oxygen species (ROS) are widely implicated in the development cardiovascular dysfunction. However, molecular mechanisms responsible for the sensitivity of the vasculature to calcium and redox imbalance and resulting dysfunctions remain elusive. We address this important issue by identifying and investigating novel mechanisms functionally linking calcium and redox signaling in arterial smooth muscle. Using a combinatorial approach including RNA interference, voltage clamp electrophysiology, and simultaneous imaging of ROS and calcium, in isolated smooth muscle cells, we find that local sites (i.e., microdomains) of calcium influx produced by L-type calcium channels colocalize and are functionally coupled with microdomains of ROS (produced by NADPH oxidase and/or mitochondria) in a bi-directional manner. Importantly, the coupling of these microdomains appears to be necessary for the contractile response of isolated vessels to the clinically-relevant vasoconstrictor angiotensin II. Finally, we are using novel approaches such as freeze fracture replica immunogold labeling (FRIL) transmission electron microscopy and other imaging techniques to characterize the subcellular architecture necessary for the functional coupling of these two ubiquitous signaling modalities.
EXPERTISE: Electrophysiology and calcium/redox signaling and imaging

38) **Endoplasmic reticulum/plasma membrane junctions function as membrane protein trafficking hubs at the neuronal perikaryon.**
PD Fox, CJ Haberkorn, AV Wiegel, EJ Akin, MJ Kennedy, D Krapf, MM Tamkun. From the Department of Biomedical Sciences, the School of Biomedical Engineering and the Department of Electrical and Computer Engineering, Colorado State University, Fort Collins, CO.
Endoplasmic reticulum/plasma membrane (ER/PM) junctions are well known for their role in store-operated Ca2+ influx via the Stim/Orai complex. We provide evidence for a novel role of ER/PM junctions as trafficking hubs for insertion and removal of plasma membrane proteins in HEK cells and neurons. By simultaneously visualizing ER/PM junctions and various transmembrane protein cargoes with total internal reflectance (TIRF) microscopy, we demonstrate that the vast majority of exocytotic delivery events for a recycled membrane protein, or for a membrane protein being delivered to the PM for the first time, occur at ER/PM junctions. Likewise, we observed stable clathrin clusters and functional endocytosis of PM proteins preferentially at EM/PM junctions. Thus, ER/PM junctions serve to organize the molecular machinery for both insertion and removal of cell surface proteins, highlighting a novel role for these unique cellular microdomains in neuronal secretory trafficking.
EXPERTISE: Microscopy, Electrophysiology, Cell culture.

39) **Monocarboxylate transporter 1 trafficking and regulation by cAMP analogs in rat brain endothelial cells.**
N Lavoy, L Li, MJ Velasquez, C Soole AL Uhernik, JP Smith. Colorado State Univ.-Pueblo, Department of Biology, Pueblo, CO
Monocarboxylate Transporter 1 (MCT1) was expressed in rat brain cerebrovascular endothelial (RBE4) cells with mCherry linked to its N-terminus. Timelapse video microscopy revealed fluorescence on the plasma membrane, in small fast moving vesicles, and in individual large donut shaped vesicles that were less mobile. In some instances the smaller vesicles moved along the inside surface of the plasma membrane, and larger vesicles sometimes appeared to interact in clusters and could occasionally be seen to exchange smaller vesicles. The large donut shaped vesicles were found in areas of low BCECF fluorescence suggesting they may be acidic and therefore possibly late endosomes or lysosomes. With constructs in which the C-terminus of MCT1 was deleted, the expression pattern was unchanged, suggesting that a PDZ ligand, WW ligand, and clathrin adaptor domain in this region were not determinants of the trafficking pattern. Confirming this, mCherry-tagged MCT1 C-terminus fusion-proteins localized oppositely, being absent from the plasma membrane and vesicles, present in the general cytoplasm and nucleus, and excluded from regions where the

large vesicles appeared with full length constructs. Intracellular pH imaging experiments showed that the mCherry-MCT1 fusion-proteins were functional, and FRAP showed that they were extremely mobile on the plasma membrane. Exposure to cAMP analogs decreased MCT1 function and caused gross morphological changes in the cells, however, the basic trafficking pattern of the fusion-proteins was preserved. Combined, these results suggest that MCT1 trafficking is highly dynamic on the plasma membrane and within intracellular vesicles in cerebrovascular endothelial cells,
and its functional regulation by cAMP analogs could involve stimulus dependent internalization by a clathrin-independent pathway leading to its degradation.
EXPERTISE: BCECF-AM intracellular pH imaging

40) **Dopamine receptor stimulation alters intrinsic resonance properties in Layer 5 pyramidal neurons in mouse medial prefrontal cortex.**
J Leyrer, J Erickson, M Spindle, M Thomas. From the School of Biological Sciences, University of Northern Colorado, Greeley, CO.
In humans, prefrontal cortical areas are known to support spatial and object-related working memory (WM) processes. In mice, working memory is mediated by homologous regions in medial prefrontal cortex (mPFC). While it is well established that WM tasks are critically dependent on optimal levels of dopamine in the PFC, the cellular mechanisms of dopamine actions are currently unknown. Studies in humans and mice have determined that WM tasks are critically dependent on generation of PFC rhythmic activity in the theta (4-7 Hz) and gamma (30-80 Hz) ranges. Rhythm generation in cortical circuits likely involves an interaction between intrinsic electrical properties and frequency-dependent synaptic properties. We are studying the intrinsic electrical resonance properties contributing to rhythmic activity in layer 5 pyramidal neurons of mPFC in the mouse, and the effects of dopamine on these resonance properties. Whole cell patch clamp recordings were performed from Layer 5 pyramidal cells in coronal slices of mouse medial PFC. We used an Impedance Amplitude Profile (ZAP) protocol to study resonance properties, using a sinusoidal current of varying frequency (1-10 Hz) applied in current clamp mode to measure voltage responses. The responses were analyzed by Fast Fourier Transform (FFT) to generate the ZAP profiles. Resonance was observed in a subpopulation of Layer 5 cells in room temperature recordings that likely corresponds to the theta range at normal brain temperatures. The presence of a hyperpolarization-activated cation current (Ih) accurately predicted whether Layer 5 neurons showed resonance, while a persistent sodium current (INa(p)) provided an amplifying influence on resonance near the resting membrane potential. Perfusion of the D1 agonist SKF-38393 significantly altered resonant frequency in a voltage-dependent manner. Experiments are currently in progress to study the effects of the D2 agonist, quinpirole, on intrinsic resonance properties. The effects of dopamine receptor stimulation may occur via modulation of the Ih and INa(p) currents, and provide a mechanism for dopaminergic modulation of rhythmic activity in prefrontal cortical circuits.
EXPERTISE: electrophysiological recordings, immunohistochemistry

41) **Using light response to trigger exocytosis from bipolar cell terminals.**
M Lipin, J Vigh. From the College of Veterinary and Biomedical Sciences, Colorado State University Fort Collins CO.
Retinal bipolar cells transmit visual input from photoreceptors to ganglion cells using graded potentials and/or spikes. The purpose of our study was to determine how graded and spiking light-evoked responses affect glutamate release from bipolar cell terminals. We used large axon terminals of ON-type, mixed bipolar cells (Mbs) in slices of dark-adapted gold fish retina. Membrane potential was recorded from the axon terminal of intact Mbs held at a resting potential equal to the threshold of action potentials in axotomized terminals. To stimulate the retina, we used 500 ms long, full-field 505 nm light flashes, with intensities between 0.5 and 100 photons/μm2/s. Then, light-evoked waveforms obtained from intact Mbs were injected into axotomized Mb terminals in voltage clamp mode in the presence of 100 μM picrotoxin. For non-spiking light responses, we injected representative waveforms recorded from the same Mb in response to different intensities. For spiking light responses, we selected a representative intact cell response to a 100 photons/μm2/s light flash containing a train of 4 spikes. For non-spiking light responses, light intensity largely affected the latency and rise time, but not the magnitude of the membrane potential change at the axon terminal of intact Mbs. Surprisingly, similar magnitudes of the light-evoked waveforms injected into axotomized terminals triggered different calcium current and exocytosis. The higher the intensity of the stimulus, the higher the rise time of the light response was, and the larger exocytosis it generated: from ~17 fF at 0.5 photons/μm2/s to ~82 fF at 100 photons/μm2/s . For spiking light responses, the triggered capacitance jump varied between~12 fF (1st spike, peaked at –34

mV) and ~77 fF (4th spike, peaked at –24.5 mV). The calcium current was negligible and did not follow the interspike waveform at holding potentials below –43 mV, but did follow it when we added 8 mV positive voltage offset to the injected waveform. For non-spiking light responses, when light intensity did not change the magnitude, the intensity was coded by the kinetics: the faster ON response triggered larger exocytosis. For spiking ON responses, when individual spikes had similar kinetics but varied in magnitude, exocytosis depended on the magnitude of spike.
EXPERTISE: Electrophysiology, patch clamp, tissue culture, animal cell culture, HPLC.

42) **μ-opioid receptor mediated modulation of intrinsically photosensitive retinal ganglion cells.**
SK Gallagher, J Vigh. Department of Biomedical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins CO.
The discovery of melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) has fundamentally altered our understanding of how light regulates mammalian physiology and behavior. ipRGCs were initially identified as a third photoreceptor type that provide light cues for the synchronization of circadian rhythms in the absence of rods and cones. Recent discoveries expand on the evolving role of ipRGCs, identifying tight interconnections within retinal circuitry and having diverse targets within discrete brain regions responsible for both circadian processing and image formation. Although ipRGCs receive rod and cone input via conventional retinal circuitry (rod/cone photoreceptors→bipolar and/or amacrine cells→retinal ganglion cells), to date only dopamine has been identified as a direct modulator of cellular function. Whether ipRGCs are targeted by neuromodulators other than dopamine remains to be explored.
The purpose of this study was to evaluate the possible neuromodulatory role of opioids on the light responsiveness of ipRGCs. Only sparse data exist for the presence or function of the μ-opioid system (the μ-opioid receptor (MOR) and its endogenous opioid peptide, β-endorphin) in the mammalian retina. Our data demonstrate MOR immunolabeling of rat ipRGCs, suggesting a functional role for the μ-opioid system in the modulation of this important cell class. To evaluate the physiological significance of MORs on ipRGCs, multielectrode array (MEA) recordings were performed using young (postnatal day 6-11) and adult (>3months) rat retinas. Systemic and direct retinal effects of DAMGO, a MOR specific agonist, on light-evoked spiking of ipRGCs were evaluated. We identified a dose dependent dramatic reduction in the light responsiveness of ipRGCs treated with MOR agonist. This MOR effect alters both the length of light response and the firing rate of ipRGCs. Such modulation of ipRGC activity could have profound consequences on light-mediated behavior and/or disease.
EXPERTISE: Immunohistochemistry and multielectrode array

43) **NF-kB activation in the hippocampus during multiple sub-threshold exposures to seizuregenic compounds.**
JA Miller, KA Sullivan, YH Raol, M Patel, RA Bialecki, RB Tjalkens. From the Center for Environmental Medicine, Colorado State University, Fort Collins, CO.
Drug-induced seizures have been documented for broad classes of pharmaceuticals including CNS and Non-CNS targeted drugs. A better understanding of the early molecular signaling events involved in promoting seizures is necessary to identify potential proconvulsive liability of new pharmacologic agents earlier in the development process. The NF-κB pathway is involved in regulating a number of stress genes and its activation may conceivably be an early indicator of potential seizure liability. Presently, we employed a NF-κB-dependent GFP reporter mouse to investigate the role of NF-κB signaling in rendering hippocampal neurons hyper-excitable. Utilizing EEG recordings and video documentation we established a sub-threshold dose level of the seizurogenic compound kainic acid (KA). Transgenic reporter mice were exposed to multiple sub-threshold doses of KA and regional and cell specific NF-κB activity was assessed after each dose. Under control levels reporter expression was absent in the hippocampus except for a slight basal expression in the CA3 pyramidal layer. Upon multiple exposures to KA, a pronounced expression of the GFP reporter was observed in the stratum moleculare, dentate gyrus molecular layer and in the dentate hilus. Additionally we exposed reporter mice to multiple low levels of a different seizurogenic compond, pentylenetetrazole (PTZ). After multiple doses of PTZ, a global increase in GFP expression was observed. Comparison of the two compounds suggests a regionally selective expression consistent with the distinct mechanisms of action for each compound. Utilizing cultured slices from the reporter mice we observed similar selective GFP expression in response to the two compounds demonstrating the potential utility of this method for the assessment of proconvulsive properties of new pharmacologic compounds.
EXPERTISE: Immunofluorescence, confocal fluorescence microscopy, qPCR, tissue culture, western blotting

44) **The role of the C_2A domain of synaptotagmin in asynchronous release.**

M Shields, N Reist. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.

Understanding the mechanisms mediating information transmission across a chemical synapse is essential to understanding brain function. During synaptic transmission, membranous vesicles within neurons are loaded with neurotransmitter, docked to the presynaptic cell membrane, primed for release, and fused with the presynaptic membrane to release neurotransmitter onto the next, or postsynaptic, cell. The vesicle membrane and proteins are recycled back into the presynaptic cell to be utilized later. There are three types of neurotransmitter release, two of which are Ca^{2+}-dependent: fast, synchronous release, and a more prolonged, asynchronous release. Asynchronous release has recently been proposed to play a role in synaptic plasticity, the basis for learning and memory mechanisms. These release processes are tightly regulated by a number of key synaptic proteins, including Ca^{2+} sensors and the fusion machinery complex. One such protein, synaptotagmin, is proposed to be the low-affinity Ca^{2+} sensor that, upon Ca^{2+} binding, triggers fast, synchronous release of neurotransmitter. In addition, studies have shown interplay between synaptotagmin and a currently unidentified and controversial high-affinity Ca^{2+} sensor responsible for the prolonged, asynchronous neurotransmitter release mechanism. At this time, it is postulated that synaptotagmin is directly inhibiting the asynchronous Ca^{2+} sensor. However, what this interplay entails is currently unknown and poorly understood. Using specific point mutations *in vivo*, the role of synaptotagmin in modulating asynchronous release will be investigated.

45) **Activity-dependent plasticity in the retina.**

RE Tooker, M Lipin, SK Gallagher, J Bramley, E Rozsa, J Vigh. From the Department of Biomedical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins CO.

Purpose: While the retina is a complex neuronal network, the only direct excitatory connection between photoreceptors and ganglion cells (GCs), whose axons comprise the optic nerve, is formed by bipolar cells (BCs). Thus, before a visual signal in the retina is transmitted to the brain, it must pass through BCs. Voltage Gated Calcium Channels (VGCCs) of BCs mediate glutamate release to GCs, and thereby the latency of the 1st action potential sent to the brain. In many CNS structures, activity-dependent modulation of presynaptic VGCCs can mediate synaptic plasticity. The objective of the present study was to elucidate whether VGCCs of BCs are subject to use-dependent modulation.

Methods: Voltage-clamp (with capacitance measurements) and current-clamp recordings were performed using Mb BC terminals in retinal slices and solitary Mbs dissociated enzymatically from goldfish retina. Light stimlation with multi-electrode array recording was used to evaluate modulation of retinal output.

Results: Strong depolarization of axotomized Mb BC terminals in a retinal slice resulted in ~5 mV hyperpolarizing shift of VGCC's activation and half-activation potential. The shift was absent with intracellular BAPTA and when evoked glutamate exocytosis from Mb BC terminals was pharmacologically inhibited. Application of ionotropic glutamate receptor (iGluR) antagonists NBQX and AP5 prevented the shift. Consistent with these results, iGluR agonists shift activation of VGCCs in axotomized Mb terminals, and increase depolarization-evoked glutamate release at physiological membrane potentials. Importantly, neither strong depolarization nor iGluR agonists altered VGCC activation in cultured, solitary Mb BCs. In slice, light could induce VGCC modulation.

Conclusion: We propose VGCCs of Mb BCs are subject to activity-dependent modulation triggered by robust presynaptic glutamate release and subsequent retrograde pathway. This sensitizes Mb terminals, increases Ca^{2+} current at physiological membrane potentials and boosts glutamate release from Mb BCs, particularly in response to weak inputs. The novel mechanism described here might contribute to the sensitization seen in some GC responses, thought to prevent visual information loss due to adaptation.

EXPERTISE: Electrophysiology, tissue culture

Neuroendocrine

46) **Mismatch of tachykinin innervation and receptors in the magnocellular portion of the paraventricular hypothalamus in rat.**

CR Brown, FW Flynn. From the Zoology/Physiology Department and Neuroscience Graduate Program, University of Wyoming, Laramie, WY.

The tachykinin family includes ligands substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), which are endogenous for the g-protein coupled receptors neurokinin 1 (NK1R), neurokinin 2 (NK2R), and neurokinin 3 (NK3R), respectively. Specifically, NK3R has a well-established and significant role in

vasopressin (VP) release from the magnocellular neurons in the paraventricular nucleus (PVN) and supraoptic nuclei (SON) in the hypothalamus. The majority of VP neurons in the PVN express NK3R, and injections of NK3R agonists stimulate VP release into circulation. Hypovolemia, and hyperosmolarity, two potent stimuli for the release of VP, cause the internalization of NK3R expressed on VP neurons. NKB is the preferred ligand for NK3R, having nearly 30 times higher binding affinity than the related ligand SP. If NKB is indeed the endogenous ligand, then there should be heavy innervation of NKB onto NK3R/VP neurons, according to typical synaptic anatomy where transmitter release sites are found directly apposed to their binding receptor sites. However, studies using double fluorescence labeling show sparse NKB innervations within the PVN, and the few terminals expressed are not found directly apposed to VP neuron somas. No reports have examined the innervation of NKB afferents onto VP dendrites, distal from the soma. What we have observed this far is that although NKB can be found around VP somas, it is sparse here in comparison to the surrounding VP dendrites. In contrast to NKB, SP densely innervates VP somas, and it has been shown that SP injections can stimulate the release of VP. However, there is still a debate on whether SP activates NK3R, as other studies have shown that SP does not directly activate NK3R under physiological conditions. Ultimately, SP has a high binding affinity for NK1R and low binding affinity for NK3R, bringing about the question of how SP plays a role in VP release from NK3R-expressing neurons. It still remains unclear how NK3R are activated in response to stimuli such as hyperosmolarity and hypovolemia, and we hope to gain more knowledge about this system by using immunohistochemical fluorescent labeling to further study the interactions between SP, NKB, VP and NK3R.
EXPERTISE: immunohistochemistry, rat femoral artery surgery, rat brain extraction and nuclear isolation, confocal imaging

47) **Clock gene mRNA expression in the paraventricular nucleus of the hypothalamus and the prefrontal cortex in male and female rats.**
LE Chun, L Woodruff, LR Hinds, RL Spencer. From the Psychology Department and Center for Neuroscience, University of Colorado, Boulder, CO.
Clock genes are molecular oscillators found in the brain and in peripheral tissues and are believed to be crucial in regulating tissue functions; disrupted clock gene expression has been linked to aberrant behaviors and physiology. Clock gene expression has been well characterized in the hypothalamic-pituitary-adrenal (HPA) axis, with peak per1 and per2 mRNA expression in the paraventricular nucleus of the hypothalamus (PVN) at Zeitgeber Time (ZT) 12-16, around the time of lights off and the onset of the active phase in rodents, and peak bmal expression at ZT4, during the rodents' inactive period. This clock gene rhythmicity in the PVN may contribute to the phase and amplitude of basal circadian peak corticosterone (CORT), which also peaks at ZT12, present in these nocturnal animals. The PVN phase of per1, per2 and bmal mRNA expression is approximately antiphasic with the expression of these genes in the suprachiasmatic nucleus. For these previous findings, only male rats were examined. Disorders associated with disrupted clock gene expression are more prevalent in females. Our present study directly compared male and female rats' per2 mRNA expression within the PVN, and CORT and ACTH plasma levels across the 24 hour day (ZTs 0, 4, 8, 12, 16, 20) in order to assess basal circadian rhythm. Using in situ hybridization and hormone assays, we were able to replicate the previous findings in males, with peak per2 mRNA expression in the PVN and CORT plasma levels (17.1 ± 3.8 µg/dL) at ZT12. We saw plasma ACTH peak (109.1 ± 13.6 pg/mL) at ZT16. The female rats were in phase with the males; their per2 mRNA expression and CORT levels also peaked at ZT12, but with peak ACTH levels at ZT12. Interestingly, while females exhibit greater amplitude of peak CORT (54.8 ± 20.5 µg/dL) and ACTH (124.1 ± 14.1 pg/mL), in line with previous studies, we did not observe a sex difference in peak per2 mRNA amplitude within the PVN (564.0 ± 49.5). Further analysis will assess the rhythmic mRNA expression of two other prominent clock genes, per1 and bmal, within the PVN and other stress-related brain regions between males and females.
EXPERTISE: in situ hybridization, behavior testing

48) **Local L-type calcium channel signaling in αT3-1 cells.**
AK Dang, NL Chaplin, C Magee, Di Murtazina, CM Clay, GC Amberg. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.
A dramatic release of luteinizing hormone (LH) from pituitary gonadotrope cells is necessary for ovulation. Binding of hypothalamic gonadotropin releasing hormone (GnRH) to its Gq-protein coupled receptor on the gonadtrope cell surface initiates multiple signaling cascades, ultimately resulting in the release of LH and induction of ovulation. We hypothesized that diacylglycerol, produced by phospholipase C, could activate

protein kinase C and stimulation of L-type calcium channels. To test this hypothesis we used a combination of TIRF microscopy and electrophysiology to image subplasmalemmal calcium influx in the gonadtrope cell line αT3-1. Using this approach we visualized discrete sites of calcium influx (calcium sparklets). METHODS: calcium influx through L-type channels was imaged with the calcium indicator fluo-5F. The external solution contained 2mM calcium. Experiments were performed at room temperature with cells voltage clamped at -70 mV. After establishing basal activity, GnRH (10 nM) was applied. For all experiments, cells were held for 1 min prior to data acquisition and images were acquired at 50 Hz. Analysis included calcium sparklet site density (sites per square micron) and calcium sparklet site activity (nPs; n is the number of quantal levels and Ps is the probability that the site is active). RESULTS: Following exposure of αT3-1 cells (n=3) to GnRH, sites of localized calcium influx were identified, whereas change sin global calcium were not evident. The L-type calcium channel antagonist nicardipine (10 μM) abolished calcium influx in response to GnRH (n=3). Conversely, the L-type calcium channel agonist FPL64176 (500 nM) produced calcium influx events indistinguishable from those induced by GnRH (n=3). CONCLUSIONS: These data suggest that GnRH activates L-type calcium channels resulting in microdomains of elevated calcium. Interestingly, the calcium signals in αT3-1 cells appear to be identical to those observed in arterial smooth muscle cells in response to the peptide hormone angiotensin II. Finally, the existence of calcium microdomains in αT3-1 cells may explain the divergent signaling cascades produced by local vs. global calcium events following GnRH receptor activation.
EXPERTISE: Electrophysiology and calcium imaging

49) **Microfluidic strategy for spatiotemporally resolved molecular sampling from live organ slices.**
C Eitel, J Wydallis, V Pauna, C Eslinger, M Mensack, DS Dandy, CS Henry, S Tobet.
From Colorado State University, Fort Collins, CO.
Advancing our understanding of organ function requires real-time, simultaneous detection of a number of key signaling molecules. In this study we implement a microfluidic device capable of sampling multiple chemical messengers from live mouse slices. In one example, A 200 μm thick organotypic ovary slice is placed on the bottom surface of a prototype sample reservoir and immersed in growth media. The bottom of the sampling reservoir contains spatially resolved 100 μm diameter sampling ports which connect to analysis microchannels, whose bottom surfaces are patterned with antibodies against specific analytes. The analysis microchannels lead to a common outlet containing multiple passive pumping reservoirs able to provide long-term, steady-state flows of a variety of complex physiological fluids. The flow rate through the analysis channels is linearly proportional to the number of pumping reservoirs in operation. Proof of principle experiments have been conducted under continuous flow conditions that demonstrate the ability of the system to accurately map the spatial distribution of multiple analytes within the sample reservoir as a function of time. This presentation focuses on the implementation of this microfluidic device, coupled with immunoassay and electrochemical detection schemes, to determine the characteristics of chemical signaling, organ development, and changes in cell behavior in response to a maturation factor such as specific steroid hormones, peptides or transmitters.
EXPERTISE: immunohistochemistry, tissue culture

50) **Visualizing cell populations with harmonic generation microscopy in the region of the paraventricular nucleus.**
JJ Field, C Eitel, KA Frahm, SA Tobet, RA Bartels. From the W.M. Keck Laboratory for Raman Imaging of Cell-to-Cell Communications, Department of Electrical and Computer Engineering, Department of Biomedical Sciences, School of Biomedical Engineering, and Department of Chemistry, Colorado State University, Fort Collins, CO.
Multiphoton laser-scanning microscopy (MPLSM) is a critical tool for examining both structure and function in various biological systems. Two-photon excitation fluorescence (TPEF) microscopy in particular has proven to be a very powerful tool for imaging numerous biological processes. Harmonic generation (HG) microscopy is an attractive compliment to TPEF as it requires no exogenous tagging to generate contrast. The structure and composition of the tissue determine the phase matching conditions for HG, and therefore the regions from which harmonic contrast is observed. Like TPEF, harmonic generation is limited to a small region near the focal plane of a tightly focused femtosecond laser pulse, leading to inherent optical sectioning and allowing for three-dimensional images to be obtained. Both the intensity and polarization state of the coherent harmonic signals carry valuable information regarding the organization and underlying structure of the specimen being imaged. Here we examine second- and third-harmonic generation (SHG and THG) in the region of the

paraventricular nucleus (PVN) in fixed murine tissues. We find that SHG and THG originate from unique populations of cells. SHG is measured from ependymal cells that line the third ventricle and form the barrier between the brain and the cerebrospinal fluid contained within the ventricle. Polarization anisotropy analysis of the SHG signal shows that the organization of the harmoniphores within the ependymal cells is complex, and points to more in-depth polarization studies which are ongoing. Conversely, THG is measured primarily from cell populations within the PVN. Since THG is sensitive to changes in the refractive index at interfaces, this suggests that these cells have unique properties that may be related to hormone production and regulation in the brain. Finally, we measure TPEF from dyes introduced through perfusion to visualize the vasculature in the region of the PVN.
EXPERTISE: Nonlinear laser-scanning microscopy

51) **Predation pressure alters stress physiology in guppies.**
EK Fischer, RM Harris, HA Hofmann, KL Hoke. From the Department of Biology, Colorado State University, Fort Collins, CO.
A central challenge for organisms faced with changing environments is coordinating phenotypic responses in multiple traits. In vertebrates, glucocorticoids mediate physiological, morphological, reproductive, immunological, and behavioral responses to stressors and are implicated in adaptive evolution following changes in predation pressure. We used the Trinidadian guppy (Poecilia reticulata) to disentangle genetic and environmental effects of predation on glucocorticoid (cortisol) levels. Guppies from high-predation environments have repeatedly and independently colonized and adapted to low-predation environments, resulting in parallel changes in life history traits, morphology, and behavior. We compared cortisol levels in two distinct evolutionary lineages to examine patterns in cortisol associated with differences in predation pressure. To distinguish genetic and environmental influences, we compared cortisol levels in guppies from different source populations reared with and without exposure to predator chemical cues. We found that fish from high-predation localities had lower cortisol levels across independent evolutionarily lineages. Evolutionary history with predators and lifetime exposure to predator cues were both associated with lower cortisol levels, but depended on distinct mechanisms. We propose that the coupling of genetic and environmental effects at a phenotypic, but not a mechanistic, level increases the flexibility and evolutionary potential of this system.
EXPERTISE: in situ hybridization, behavior testing, immunohistochemistry

52) **Localization of a gonadotropin-releasing hormone like-molecule in a gastropod mollusk, Aplysia californica.**
L Jung, SI Kavanaugh, P-S Tsai. From the Department of Integrative Physiology, University of Colorado at Boulder, Boulder, Colorado.
Gonadotropin-releasing hormone(GnRH) play important roles in vertebrate reproduction. Recently, molecules structurally similar to vertebrate GnRH were discovered in a gastropod mollusk, Aplysia californica. To infer their reproductive involvement in an invertebrate, the present study examined the localization of Aplysia GnRH (ap-GnRH) transcripts in the central tissues of A. californica. Further, we authenticated the expression of the former with ap-GnRH immunohistochemistry. Reverse transcription polymerase chain reaction (RT-PCR) revealed the ubiquitous expression of ap-GnRH in the both peripheral and central tissues including the osphradium, tail, bag cell neurons, small hermaphroditic duct, and abdominal, cerebral, and pedal ganglia. However, in situ hybridization (ISH) detected ap-GnRH transcript only in the pedal (ca. 17 neurons), cerebral (ca. 10 neurons), and abdominal (ca. 4 neurons) ganglia, suggesting RT-PCR may have detected biologically insignificant levels of ap-GnRH gene expression in the peripheral tissues. ap-GnRH IHC revealed that most neurons positive for ap-GnRH transcripts were also positive for the ap-GnRH peptide. Overall, our data suggest that ap-GnRH is widely distributed across central ganglia and support the notion that ap-GnRH may assume multiple functions beyond reproduction.
EXPERTISE: In situ hybridization, immunocytochemistry, and paraffin embedding

53) **Chronic glucocorticoid intake abolishes the diurnal pattern of slc6a4 and htr1a mRNA expression in anxiety-related serotonergic systems.**
S Mani, NC Donner, AJ Matti, CA Lowry. From the Integrative Physiology Department and Center for Neuroscience, University of Colorado, Boulder, CO.
Research has demonstrated that the diurnal expression pattern of tph2, the gene encoding the rate-limiting enzyme during serotonin (5-hydroxytryptamine, 5-HT) synthesis, depends on the circadian glucocorticoid (GC) rhythm. Research from our lab has established that chronic GC exposure causes an anxiety- and depression-

like phenotype associated with an abolished diurnal variation of tph2 expression due to increased tph2 expression during the inactive phase (light phase in rodents). We here hypothesized that slc6a4, the gene encoding the serotonin transporter (SERT), and htr1a, the gene encoding the autoinhibitory 5-HT1A receptor, are also expressed in a diurnal pattern that is altered by chronic GC exposure. To test this hypothesis, we subjected adrenal-intact adult, male rats to 40, 100, or 400 µg/ml corticosterone (CORT) or vehicle (0.45% 2-hydroxypropyl-β-cyclodextrin) via the drinking water for 21 days, and assessed slc6a4 and htr1a mRNA expression within the serotonergic dorsal raphe nucleus (DR), the main source of brain 5-HT, using in situ hybridization histochemistry. We found that CORT-treatment specifically affected slc6a4 and htr1a mRNA expression within specific DR subregions. In vehicle-treated rats, the anxiety-related dorsal DR (DRD) displayed a diurnal variation in slc6a4 expression that was abolished in CORT-treated rats by suppressing slc6a4 expression dose-dependently during the rats' active dark phase. CORT treatment also decreased dark-phase slc6a4 expression in the caudal part of the ventral DR (cDRV). Expression of htr1a occurred in a diurnal pattern within the anxiety-related caudal DR (DRC), a pattern that was, again, abolished by chronic CORT treatment. No other subdivisions displayed a diurnal expression pattern of either gene, suggesting an indirect effect of chronic CORT treatment on gene expression, possibly via alteration of afferent signaling from the extended amygdala complex. The observed changes indicate an overactive serotonergic system (more 5-HT production, less 5-HT reuptake, and disrupted diurnal 5-HT1A -mediated autoinhibition) particularly within those DR subdivisions previously associated with control of anxiety-related behavior.

54) **Fibroblastic growth factor signaling deficiencies exerted variable age-dependent effects on SCN VIP neurons of male mice.**

AV Miller, SI Kavanaugh, MA Basse, SD King, PS Tsai. From the Department of Integrative Physiology, University of Colorado Boulder, CO.

Fibroblastic growth factor (Fgf) 8 orchestrates the development of the nose, telencephalon, midbrain, and hindbrain. However, its role in the development of structures that originate within the diencephalon was unclear. The goal of the present study was to understand if deficiencies in Fgf8 and one of its cognate receptors, Fgfr1, disrupted the organization of the suprachiasmatic nucleus (SCN), a hypothalamic nuclei originating within the diencephalon and critical for circadian integration and output. Immunohistochemstry (IHC) of a SCN neurochemical marker, vasoactive intestinal peptide (VIP), was conducted on wild type (WT), Fgf8 heterozygous hypomorph (F8 HET), Fgfr1 heterozygous knockout (R1 HET), and Fgf8/Fgfr1 double heterozygous (DH) mice. Brains from each of these genotypes were dissected on postnatal days (PN) 0, 30, 60, or 90, fixed, sectioned, and immunostained for VIP. On PN0, Fgf8 hypomorphy led to a significant reduction in the number of SCN VIP neurons in mixed-sex pups, although the effects of Fgfr1 deficiency and DH were not examined. From PN30-PN90, different Fgf signaling deficiencies exerted variable age-dependent effects on SCN VIP neurons of male mice. Specifically, WT and F8 HET males had constant numbers of SCN VIP neurons from PN30-PN90. R1 HET males had higher SCN VIP neuron on PN30, but this number declined to normal on PN90. Lastly, the number of SCN VIP neurons in DH males was normal on PN30, but underwent a progressive increase as they aged. By PN90, the numbers of SCN VIP neurons were similar among WT, R1 HET and F8 HET, but significantly increased in DH males. In sum, our data suggest that the impact of Fgf signaling deficiency persists beyond the developmental period into adulthood. In this regard, Fgf signaling deficiencies likely impact the synthesis of VIP at the transcriptional or post-transcriptional level instead of irreversibly altering the developmental fate of these neurons. Lastly, combined deficiencies in Fgf8 and Fgfr1 may trigger compensatory responses that lead to the abnormal increase in SCN VIP neurons through unknown mechanisms. (Supported by NIH R01 HD042634).

EXPERTISE: Immunohistochemistry

55) **Endocannabinoid signaling as an intrinsic component of the circuits mediating adaptive responses to repeated stress exposures.**

RJ Newsom, CV Masini, TJ Nyhuis, HE Day, S Campeau. From the Department of Psychology, University of Colorado-Boulder, Boulder, CO.

Evidence implicates the endocannabinoid (eCB) system as a negative modulator of neural and endocrine responses to acute stressors. Recently, eCBs were also shown to influence the development of habituated hypothalamo-pituitary-adrenal (HPA) axis responses to repeated homotypic stress, in which repeated exposures to the same stressor reliably decreases subsequent activation of the HPA axis. The present studies were initiated to distinguish a potential role of eCB signaling in the acquisition as compared to the expression of habituated HPA axis responses. Adult male Sprague Dawley rats (Harlan) were exposed to daily, 30 minute

sessions of loud white noise (95 dB) for 8 days. Rats received either the cannabinoid receptor 1 (CB1) antagonist AM251 (2mg/kg or 0.5mg/kg, i.p.) or vehicle 30-min before the loud noise sessions on the first 7 days, but not on the 8th day of stress exposure. The rats treated with 2 mg/kg AM251 had higher levels of plasma corticosterone (CORT) on days 1,4, and 7. However, on day 8 of noise (drug-free), 2 mg/kg AM251-treated rats had near complete attenuation of HPA axis response, as observed in rats receiving vehicle or 0.5 mg/kg AM251 injections during the initial 7 loud noise exposures. This suggests that disruption of CB1 receptor signaling does not disrupt the plasticity associated with acquisition of habituation to repeated homotypic stress. In a second study, rats were exposed to daily loud noise stress for 7 days to establish HPA axis habituation without drug treatments. Twenty-four hrs later, rats were injected with 1 mg/kg AM251 or vehicle, 30 min prior to a final 30-min loud noise exposure. Vehicle-treated rats displayed reliable habituation of HPA axis response, but CB1 antagonism disrupted the expression of this habituated response, by restoring plasma CORT to levels observed during the initial (day 1) loud noise exposure. Rats were given an additional mild stress exposure (novel environment) 24-hours later, along with a cohort of rats not previously habituated to noise stress. 1 mg/kg AM251 treatment prior to novel environment resulted in a significantly larger HPA axis response, but only in rats recently habituated to repeated loud noises.

56) **Reversible inactivation of the rostral raphe pallidus disrupts acute autonomic responses but not habituation to repeated loud noise stress exposures in rats.**

CV Masini, TJ Nyhuis, HEW Day, S Campeau. From the Department of Psychology and Neuroscience, University of Colorado Boulder.

Evidence indicates that the medullary rostral raphe pallidus (rRPa) mediates several autonomic responses to stress exposure including tachycardia and thermogenesis. This area of the rRPa shows robust Fos induction following audiogenic stress exposure, which also induces an increase in heart rate and body temperature acutely. The present study was designed to assess the possibility that the rRPa, due to its known control of tachycardic and hyperthermic responses following acute stress exposure, may contribute to the plasticity associated with habituation of these responses to repeated loud noise exposures. Adult male rats were implanted with indwelling cannulae in the rRPa and telemetric E-mitter transponders (Mini Mitter, Sunny River, OR). After recovery from surgery, animals were transported to testing chambers and their home cages were placed on ER-4000 Energizer receivers. Following an acclimation period in the chambers, the rats were given three daily injections (200 nl) of muscimol (1 mg/ml over 1 min) or artificial cerebral spinal fluid, followed by 30 minutes of 95-dBA loud noise exposures. Twenty-four hours following the third noise exposure, animals were handled and exposed to an additional drug-free 95-dBA noise test. Two days following the final noise exposure, animals were given a 30-minute restraint stress exposure within the testing chambers, in order to evaluate their ability to display normal acute stress responses. In rats with verified placements in the rRPA (dye injections), results suggest that rRPa muscimol reliably attenuates acute noise-induced tachycardic and thermogenic responses, compared with aCSF-injected rats. Interestingly, animals in both treatment groups exhibited similar habituation of heart rate and body temperature during the drug-free test noise exposure. All animals displayed similar robust heart rate and body temperature increases to the subsequent acute restraint test. These results indicate that the rRPa is not a site of plasticity associated with habituation of the autonomic responses following repeated stress.

57) **Reversible inactivation of a subregion of the posterior hypothalamus disrupts HPA axis habituation to repeated stress exposures in rats.**

TJ Nyhuis, CV Masini, RJ Newsom, HEW. Day, S Campeau From the Department of Psychology and Neuroscience, University of Colorado Boulder.

The medial parvicellular nucleus of the paraventricular hypothalamic nucleus (mpPVN) is known to mediate activation of the neuroendocrine hypothalamo-pituitary-adrenal (HPA) axis, which leads to elevated plasma corticosterone (CORT) levels in response to stress. Similarly, the rostral region of the raphe pallidus (rRPa) mediates a number of stress-induced autonomic responses, including tachycardia and hyperthermia. In turn, both the mpPVN and rRPa are innervated by a specific region of the posterior hypothalamus (PH), many neurons of which express stress-induced FOS induction. Audiogenic stress induces increases in HPA axis activity, heart rate and body temperature. The current study was designed to assess the possibility that the PH, due to its projections to both the mpPVN and rRPa, contributes to the control of multiple responses associated with acute and repeated audiogenic stress exposures. Adult male Sprague-Dawley rats were implanted with indwelling bilateral cannulae in the anterior portion of the PH. Following recovery from surgery, animals were given three daily injections (200 nl) of muscimol (0.5mg/ml) or artificial cerebral spinal fluid (aCSF; vehicle),

followed by 30 minutes of 95-dBA loud noise exposures. Fourty-eight hours following the third noise exposure, animals were handled and exposed to an additional drug-free noise test. Corticosterone was measured from plasma collected immediately following the first and test noise exposures. In rats with verified cannulae placements, muscimol reliably attenuated the acute plasma CORT response to loud noise as compared to vehicle-injected animals. Furthermore, in response to the drug-free loud noise test vehicle-injected rats displayed a reliable habituation of CORT, while CORT response habituation was attenuated in muscimol-injected rats. These results suggest that the anterior portion of the posterior hypothalamus significantly contributes to the acute HPA axis response to stress and its habituation following repeated homotypic stress exposures. Current studies are underway to determine the role of the PH in acute autonomic responses to audiogenic stress and their habituation following repeated homotypic stress exposures.
EXPERTISE: immuno/ in situ

58) **Corticosterone treatment immediately after stress onset produces a delayed negative feedback effect on HPA axis activity.**

C Osterlund, M Rodriguez, R Spencer. From the University of Colorado-Boulder, Boulder, CO.

A phasic increase in corticosterone (CORT) secretion suppresses stress-stimulated hypothalamic-pituitary-adrenal (HPA) axis hormone release through mechanistically distinct protein synthesis independent or dependent temporal domains that are referred to as fast or intermediate CORT negative feedback. Studies in the 1960s and 1970s established the existence of CORT negative feedback control of the HPA axis. Most of those studies relied on indirect measures of hormone levels, and the in vivo studies were typically conducted under non-physiological conditions. Consequently, much of this research has provided little clarity about which intercellular and intracellular HPA responses during stress are altered by a phasic rise in CORT. We tested the ability of phasic CORT (0.3mg/kg) prestress onset treatment (1h, 15min, or 30s) or 5min poststress onset treatment to suppress HPA axis adrenocorticotropic hormone (ACTH) and prolactin response to an acute psychological stress challenge (restraint 30m). We surgically implanted jugular catheters into young adult male Sprague-Dawley rats (N=72). Our findings show that stress evokes prolactin release and CORT treatment before or after the onset of stress did not inhibit this release. This suggests that CORT negative feedback must be taking place at the intrinsic level of the HPA axis, and that stimulated prolactin release is not subject to CORT regulation. In contrast, we determined that CORT administered 1h, 15min, or 30s before stress onset substantially blunted stimulated ACTH secretion. However, CORT administered to adrenal intact rats 5min following the onset of stress caused no additional suppressive action over stimulated ACTH secretion, suggesting that CORT is unable to produce a fast feedback effect after the onset of stress. Testing this theory, we removed endogenous CORT activity and selectively reinstated CORT during an ongoing stress response. As a result reinstatement of CORT produced a delayed suppressive effect over stimulated ACTH secretion. These studies indicate that the temporal window for the induction of fast feedback by CORT is longer than previously believed if CORT increase precedes stress onset (extends to 15 min), but is attenuated and delayed if CORT increase follows stress onset.

59) **Characterization of glucocorticoid modulation of clock gene oscillation in the frontal cortex.**

L Woodruff, LE Chun, M Girotti, LR Hinds, RE Ramsey, C Osterlund, RL Spencer. From the Department of Psychology and Neuroscience, Unversity of Colorado, Boulder, CO.

Daily circadian patterns of glucocorticoid receptor (GR) activation are able to modulate rhythmic clock gene expression within the periphery and there is support for a similar effect on Per2 protein levels in some subcortical brain regions. Thus it appears that the master circadian clock housed within the hypothalamic suprachiasmatic nucleus (SCN) is able to synchronize extra-SCN molecular clocks at least in part by controlling the circadian release of corticosterone (CORT). Altered CORT secretion, such as that caused by chronic stress may lead to disrupted clock gene expression within various brain regions such as the prefrontal cortex, and that may contribute to the abnormal neuroendocrine and emotional control associated with stress-related disorders. Many peripheral clocks have been found to be modulated by altered CORT secretion, yet to date virtually no characterization of such has occurred in the prefrontal cortex. In preliminary studies we observed a robust rhythmic clock gene expression in frontal cortex (per1, per2, and bmal1 mRNA). We now aim to determine to what extent this expression is modulated by CORT circulation patterns in adult male Sprague Dawley rats (270-300g). Animals received either sham or adrenalectomy (ADX) surgery. Animals were then further divided into three groups: Sham/vehicle injection (n=24), ADX/vehicle injection (n=24), and ADX/CORT injection (n=24). All injections occurred at ZT1, the time during which endogenous CORT is at its lowest in adrenal-intact nocturnal rodents. Rats were then sacrificed at 4 zeitgeber times (ZT0, ZT6, ZT12, and

ZT18). Brain and tissue samples were analyzed via in situ hybridization for the expression of per1, per2, and bmal1 mRNA. Plasma samples were assayed for ACTH and corticosterone levels. We have shown previously that per1 and per2 expression in the frontal cortex peaks during the dark period, and bmal1 expression peaks during the light period, indicating the baseline functioning of the positive and negative arms of the oscillatory mechanism. Ongoing work will characterize the extent to which CORT circulation modulates the profile of clock gene expression within the prefrontal cortex.
EXPERTISE: In situ hybridiation, enzyme linked immunosorbent assay, radioimmuno assay

Sensory and Motor Systems

60) **Applying mutual information theory to the auditory system.**
K Anbuhl, D Tollin. From the Department of Physiology & Biophysics and the Neuroscience Training Program, University of Colorado School of Medicine, Aurora, CO.
In young children, recurring ear infections producing a conductive hearing loss (CHL) can result in persistent problems with speech perception, as well as an inability to accurately localize sounds in noisy environments. Studies have shown that this impairment in sound location ability is due to altered interaural level difference (ILD) cues, which are used to locate the source of high frequency sounds. It is not known, however, how CHL alters the neural information-carrying capabilities of the auditory system. To address this question, we propose to use the mathematical framework of mutual information theory to assess information processing in the auditory system of the chinchilla. If early CHL reduces the information coding capabilities of neurons sensitive to ILD cues in any way, mutual information theory will capture this trend. We expect to see a reduction in the neural information processing in animals exposed to CHL, and this may explain the problems with binaural hearing abilities observed clinically in children.

61) **Peripheral anatomy of two classes of slowly adapting vibrissal sinus hair follicle afferents.**
CM Cassidy, CJ Woodbury. From the Graduate Neuroscience Program and the Department of Zoology and Physiology, University of Wyoming, Laramie, WY.
Sinus hair follicles (SHFs) are complex sensory organs that have dense and highly stereotypical innervation patterns comprised of a number of anatomically distinct nerve terminals. Although many theories have been proposed about the functional properties of each afferent ending, little conclusive evidence for these theories exists. To determine the terminal morphology of physiologically identified afferents innervating SHFs, we used an in vivo mouse preparation to study both the physiology and peripheral anatomy of a single primary afferent. Intrasomal recordings were obtained from the trigeminal ganglion of decerebrate, paralyzed, and artificially ventilated adult mice. After physiological characterization, neurons were iontophoresed with Neurobiotin for subsequent analysis of peripheral terminals. Here, we describe the peripheral anatomy of two classes of slowly adapting afferents innervating SHFs. Both sets of afferents show irregular patterns of discharge throughout maintained stimuli. In the first set of afferents (n=15), the axon gives rise to fine, localized, terminal branches that fan out to form a grapelike cluster of flattened expanded discs that are highly reminiscent of the tactile discs associated with Merkel cells in touch domes of generalized hairy skin (Iggo & Muir, 1969). The second set of afferents (n=3) terminate as longitudinal palisades of lanceolate endings. The peripheral anatomy of the first set is unsurprising as Merkel cell-neurite complexes have been shown to exhibit slowly adapting type I responses in touch domes in normal hairy skin (Woodbury & Koerber, 2007). The peripheral anatomy of the second set of afferents is wholly unexpected. The anceolate endings terminating on SHFs are widely assumed to be rapidly adapting, a finding that was recently confirmed in generalized hairy skin (Li et al., 2011). Our present findings help answer longstanding questions on the functions of structurally distinct afferent endings associated with SHFs and illustrate that physiology cannot be dictated solely on the basis of terminal morphology. Future work will continue to aid in elucidating the structure/function relationships of anatomically specialized neuronal endings in the skin.

62) **Peripheral color vision can be better than foveal color vision.**
JK Opper, ND Douda, VJ Volbrecht, and JL Nerger. From the Department of Psychology, Colorado State University, Fort Collins, CO.
The presence of three cone types in the retina is required to mediate trichromatic vision and perceive color. Because the central focal point of the retina, the fovea, is comprised of only cones, it is assumed that color perception in the fovea is more discriminating than color perception in the peripheral retina, which is comprised of both rods and cones. Rods are known to degrade color perception by reducing the amount of hue perceived

in an object (i.e., a red stimulus will become pinkish). Increasing the size of the stimulus in the peripheral retina, however, can reduce these rod effects until color perception becomes “fovea-like”. The assumption is that even if peripheral color perception becomes “fovea-like” it will never exceed the color perception capabilities of the fovea. In this study color-naming data were acquired for 23 monochromatic stimuli, ranging from 440-660 nm, presented to the fovea and at 10° retinal eccentricity. Stimuli in the peripheral retina were of the appropriate size to generate “fovea-like” color perceptions under two conditions: a light-adapted condition to minimize rod input and dark-adapted condition to maximize rod input. In one experiment the same peripheral location was used but stimuli varied in retinal illuminance while in the second experiment stimuli were at the same retinal illuminance and presented at four different peripheral locations in the nasal, temporal, superior, and inferior retinas. For two of three observers in each experiment, saturation (i.e., chromatic content) was unexpectedly greater for some of the middle- and long-wavelength stimuli in the peripheral retina than in the fovea under some experimental conditions. This finding is contrary to the idea that foveal color perception is always more acute than peripheral color perception and suggests that rods may not necessarily contribute to the suppression of chromatic neural processing, but may actually enhance it under certain situations. Supported by NSF grant (1127711) to VJV and JLN.

63) **The Nuclei of the lateral lemniscus: Cytoarchitecture and connectivity with the inferior colliculus.**
DR Pitynskil, ZM Fuzessery. From the Neuroscience Program, University of Wyoming, Laramie, WY.
The nuclei of the lateral lemniscus (NLL) have not been previously studied in the pallid bat (Antrozous pallidus). The columnar region of the ventral NLL (VNLLc) has been found only in echolocators. Here we ask 1) if the VNLLc is present in the pallid bat, 2) what is its relationship to surrounding nuclei, 3) if the VNLLc are found only in echolocators, does it process only echolocation, and 4) what is the tonotopic organization within the NLL? The pallid bat is a gleaning bat that captures prey items from terrestrial matter. To do this the pallid bat uses passive sound localization to find prey, but uses echolocation to navigate its environment. These two functionally distinct systems are also divided within the pallid bat IC. Using fluorescent retrograde tracers we injected into the echolocation (40kHz) and the low frequency, passive sound localization (15kHz) areas of the IC. Our results show that the pallid bat does have a VNLLc, and it is located dorsal to the multipolar region of the VNLLm. The VNLLc received projections from a low frequency, non-echolocation area, indicating that it processes both non-echolocation and echolocation, and the VNLLc possesses a clear tonotopic map, but the remaining NLL in the pallid bat does not.

64) **Differential distribution of AgRP and POMC in taste buds of mice.**
SH Monahan, ST Hentges, LM Stone. From the Biomedical Sciences Department, Colorado State University, Fort Collins, CO.
In a healthy individual, circuits in the oral cavity, digestive system and central nervous system work together to maintain homeostatic balance between nutrient intake and energy expenditure. Disruption of these circuits can lead to obesity and associated diseases, which are increasing global health issues. In the oral cavity, changes in the palatability of foods due to changes in taste receptor responses likely influence feeding behavior. Recent evidence indicates that hormone and peptide receptors are present in subpopulations of taste cells and activation of these receptors leads to changes in taste sensitivity. Specifically, taste cells express neuropeptide Y (NPY, Zhao et al., 2005) and receptors for endocannabinoids and leptin receptors (for review see Niki et al., 2010). These proteins have been studied extensively in the arcuate nucleus of the hypothalamus, and are present in two main opposing circuits that regulate energy balance. Activation of the first circuit results in in an increase in feeding. Cells within this orexigenic circuit express NPY and agouti gene-related peptide (AgRP). The opposing circuit is anorexigenic and contains cells that express proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). In the current study, we use immunocytochemistry and POMC-GFP mice to show that subpopulations of taste cells express AgRP and POMC. AgRP is expressed in circumvallate and foliate taste buds, but is rarely observed in fungiform taste buds. AgRP and the G protein α-gustducin are co-expressed in some taste cells, but T1R3 positive taste cells lack AgRP-ir. This suggests that AgRP is associated with bitter-sensitive type II taste cells, or with type III taste cells. Taste buds from POMC-GFP mice exhibit GFP-ir indicating that POMC peptides are also expressed in taste buds. POMC-GFP is present in both gustducin-ir cells and in cells that lack gustducin. Thus POMC is expressed in at least 2 populations of taste cells including some type II cells. In summary, taste receptor cells express peptides that are involved with maintaining energy homeostasis in the hypothalamus. Taste cells expressing these peptides likely have roles in regulating energy balance by affecting food palatability.
EXPERTISE: immunocytochemistry, confocal microscopy

65) **Cholesterol depletion activates TRPV1.**

B Surenkhuu[1,#], P Baskaran[2,#], S Clark[1,2], B Culver[1,2], B Thyagarajan[1,2,*]. From the [1]Program in Neuroscience and [2]School of Pharmacy at the University of Wyoming, Laramie. [#]Contributed equally; *corresponding author.

Transient receptor potential vanilloid receptor 1 (TRPV1), the capsaicin receptor, is a non-selective cation channel activated endogenously by noxious heat, pH and anandamide and exogenously by capsaicin and resineferatoxin. Here we investigated the effects of cholesterol depletion on TRPV1 activation by capsaicin or acidic pH (pH 5.5). Membrane cholesterol perturbation was achieved by pretreating cultured TRPV1 stably expressing HEK293 cells with either methyl-β-cyclodextrin (MβCD, chelates cholesterol; 10 mM; 1 hr at 22 °C) or atorvastatin (ATS, HMG CoA reductase inhibitor; 10 μM; 4 hr at 37 °C). Both MβCD and ATS activated capsaicin or pH 5.5-stimulated Ca2+ influx via TRPV1. In the absence of extracellular Ca2+, MβCD pretreatment significantly increased capsaicin-stimulated Na+ currents into HEK TRPV1 cells. MβCD pretreatment resulted in increased expression levels of TRPV1 and PKC as measured by immunoblotting. Pretreatment of HEK TRPV1 cells with αCD, an agent with a structure similar to that of MβCD but lacking the ability to deplete membrane cholesterol, did not affect TRPV1 activity. Our data indicate that perturbation of membrane cholesterol activates TRPV1 by via PKC-dependent phosphorylation of TRPV1 as well as by increasing the expression levels of TRPV1. Further work is in progress to identify the mechanism by which cholesterol rich lipid microdomains (LMD) regulate TRPV1 activity. Acknowledgment: 1001070G NIH/DHHS COBRE (B Thyagarajan)1001481H NIH/DHHS INBRE (B Thyagarajan)International Students Office Scholarship, & Bruce Culver, PhD (B Surenkhuu)

EXPERTISE: Cell culture & intracellular calcium imaging

66) **Activity in the mouse pedunculopontine tegmental nucleus correlates with action selection and execution.**

JA Thompson, Gidon Felsen. From the Department of Physiol. and Biophysics, Univ. of Colorado Denver Anschutz Med. Campus, Aurora, CO.

Accrued neurophysiological evidence in rat, cat, and non-human primate has identified a network of brain areas that contribute to the process of decision making, from acquisition of sensory evidence to response selection (Gold and Shadlen, 2007). One such region that has received relatively little attention is the pedunculopontine tegmental nucleus (PPT) located in the caudal pontomesencephalic tegmentum, which innervates the basal ganglia, superior colliculus, thalamus and cortical targets via cholinergic and glutamatergic projections (Steckler et al., 1994, Jenkinson et al., 2009). While the PPT has most often been studied in the context of controlling behavioral arousal, these hodological and neuromodulatory properties place the PPT in an integral position to control motor output. Recent studies implicate the PPT in preparing and executing movements. In order to examine the role of the PPT in these processes, we performed tetrode recordings in the PPT of mice engaged in a sensory-cued, two-alternative forced-choice delayed-response task. In each trial of the task, the mouse sampled an odor at a central port and, depending on the identity of the odor, prepared to move to the left or right reward port to receive a water reward. Movement was initiated following the presentation of a “go signal”, 450-600 msec after stimulus onset. We examined neural activity during several epochs of the task related to the preparation and execution of the movement. We observed that a substantial proportion of neurons exhibited elevated activity during one or more of these epochs, often in a direction-selective manner. These results suggest that that the PPT is part of the network of brain regions responsible for sensorimotor decision making. Given the heterogeneity of neural phenotypes and complex connectivity of the PPT, in future experiments we will examine how specific classes of PPT neurons contribute to motor control.

67) **Balancing excitation and inhibition within olfactory bulb glomeruli.**

JD Zak, DH Gire, NE Schoppa. From the Department of Physiology & Biophysics, University of Colorado Anschutz Medical Campus, Aurora, CO.

A key cellular element that controls the output of glomeruli in the mammalian olfactory bulb are a class of glutamatergic cells that surround glomeruli, the external tufted (ET) cells. Glutamate released from ET cells can excite the output mitral cells (MCs) as part of a feedforward excitatory path from olfactory sensory neurons (OSNs), while they can also excite GABAergic periglomerular (PG) cells that in turn inhibit MCs. To examine factors that determine whether ET cells drive net excitation or inhibition of MCs, we performed patch-clamp recordings from identified neurons in rat olfactory bulb slices. We found in this analysis that the balance depended profoundly on the strength of excitation of ET cells. During ET-MC pair-cell recordings, excitatory

currents in MCs were very small when ET cells engaged in only single spikes, which contrasted with rather large unitary excitatory post-synaptic currents (EPSCs) that were measured in PG cells. The much smaller ET-to-MC current, which would favor net inhibition, reflected the fact that ET-to-MC signaling, unlike ET-to-PG cell signaling, occurs via long-range diffusion of glutamate. On the other hand, two mechanisms favored excitation of MCs when ET cells were more strongly excited. One of these was simple accumulation of glutamate within the glomerulus with repeated ET cell spiking: during ET-MC pair recordings, spike barrages in ET cells could cause large MC currents. A second factor was activation of metabotropic glutamate receptors (mGluRs), which reduced GABA release from PG cells. This effect was seen as a reduction in the disynaptic inhibitory current in ET cells caused by the group II mGluR agonist DCG-IV, as well as an increase in inhibitory currents driven by direct ET cell stimulation due to antagonists of group II mGluRs. DCG-IV also increased the probability of the glomerulus-wide long-lasting depolarization evoked by OSN stimulation, directly demonstrating that mGluR activation can shift the balance of glomerular activity toward excitation. Taken together, these results indicate that the balance of excitation and inhibition in glomeruli is controlled in a stimulus-dependent fashion by the dynamics of glutamate diffusion within a glomerulus and metabotropic glutamate receptors.
EXPERTISE: electrophysiology

Teaching

68) **Educational opportunities for CSU students and local high school students during Brain Awareness Week.**

LS Baker, LM Stone. From the Psychology Department and the Department of Biomedical Sciences at Colorado State University, Fort Collins, CO.

Brain Awareness Week is a program designed to introduce neuroscience and brain research to the public. This global program is supported by the Society for Neuroscience and the Dana Foundation. Locally in Fort Collins, this program is supported by the Front Range Neuroscience Group and made possible by numerous volunteers from Colorado State University. We have been involved with this program for over 10 years and successfully provide educational opportunities to both CSU students and to local high school students. CSU students are trained to present neuroscience material at selected Fort Collins high schools, giving them valuable teaching experience in a small group setting. Local high school students are able to interact with CSU volunteers and learn about neuroscience, research and neuroscience careers. The physical core of our program is a set of demonstration stations that include both a poster on a specific topic and some associated activities to help the high school students understand key concepts. We have stations covering topics including but not limited to, neuroanatomy, epilepsy, sensory systems and synaptic transmission. In addition to the station materials, we have a core group of CSU volunteers that coordinate the event each year. However, we also need new volunteers each year to help with presentations and encourage those who are interested to contact us. In addition, we will help formulate a plan for individuals interested in providing a Brain Awareness Week program in their local communities.

69) **From the pupil's perspective: A look at the benefits outreach presentations offer pre-professional students**.

AH Beach, LL Leach, TR Clapp. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.

We suggest university anatomy outreach programs promote the personal and professional development of student presenters better preparing them for healthcare professions. University student-led anatomy outreach programs presented to high school students provide an opportunity to develop unique skills that better prepare the university students to be successful future health care professionals. Some of these skills include time management, professional communication, scheduling, and working with emotional barriers. Importantly, delivering these presentations requires students to acknowledge and address their own weaknesses. Interacting with a variety of audiences, the university student presenters also learn to accommodate and adapt to varying knowledge levels. Ultimately this better prepares them to convey difficult scientific information to a wide variety of individuals. These outreach programs benefit presenters and audiences allowing students to share and apply their knowledge and enthusiasm as well as encourage an interest in science.
EXPERTISE: Teaching, education, human gross anatomy, neuroanatomy

70) **Analyzing neuroscience educational approaches and success.**
M Mesiha, S Hentges. From Colorado State University, CO.
Classes that present information at the cellular level often facilitate learning with laboratory or recitation components. When neither is practical, a simulation program may be used. Neurons in Action 2 (NIA) contains tutorials that explore neuron structure and function. A survey distributed to 94 Cellular Neurobiology students at Colorado State University sought to analyze correlations between NIA use, neuroscience interest, and academic performance. Students who indicated neuroscience interest comprised 43% of NIA users, although 40% of those fulfilling a graduation requirement used NIA. NIA was disregarded by 55% of students interested in neuroscience possibly due to a superior understanding of the material.NIA users display better academic performance with 44% receiving A's and B's as well as an 11% grade improvement from the first exam to the second. Students who chose different study methods demonstrated a 26% grade improvement, but only 23% indicated scoring A's and B's. Those interested in neuroscience scored the highest on exams with 38% scoring A's and B's on both exams. Students with other interests including post-graduate plans showed 35% of A's/B's on exams with a 30% grade improvement. Students without neuroscience interest and lack of NIA use demonstrated poor academic performance. Survey results revealed that students solely interested in neuroscience as well as NIA users perform better academically. Extensive incorporation of NIA into lecture and a recitation or laboratory component should be added to the course to aid in visualization of difficult concepts.

71) **The use of non-prescribed Adderall and Ritalin in a collegiate setting.**
ML Miller, TR Clapp. From the Department of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.
Adderall (Dextroamphetamine) and Ritalin (methylphenidate) are commonly prescribed medications for patients suffering from attention deficit, hyperactive disorder or ADHD. A common side effect of Adderall and Ritalin use is the increased attention and focusing abilities of the user, due to increased dopamine levels. The purpose of this study was to investigate the relative use of Adderall and Ritalin among active students of Colorado State University through anonymous surveys of the student body. A total of 665 surveys were completed with 143 students indicating current or past use of Ritalin, Adderall or a combination of both without a prescription. Out of the 143 students, 107 (75%) indicated preference for Adderall consumption while 28 (20%) used both Ritalin and Adderall and 7 participants (5%) indicated Ritalin use. Though indicated reasons for the non-prescribed use varied widely, there was a common thread of studying for exams in the majority of the surveys (N=107). Interestingly, most users of Adderall and Ritalin knew of the prescription medication status of both drugs, and intend on pursuing professional school admittance. The data also suggests an increase in Adderall and Ritalin use by upperclassmen (junior and senior level). These survey results represent the use of Adderall and Ritalin in less than 3% of the total student body.
EXPERTISE: Gross human anatomy, gross neuroanatomy, education modules, drug use, human survey studies, IRB project development

72) **The case study: Figuring out how to figure it out.**
A Vaudreuil, TR Clapp. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.
There is a currently trend in higher education toward problem-based learning. One strategy to introduce problem based learning is through the use of case studies. With the use of case studies students are forced to critically read through a wealth of information drawing out the important information and while wading through the many distracters. Many times data can be incomplete forcing students to look for information elsewhere. We like to call this process of discovery "figuring out how to figure it out". In other words students gain knowledge on how to find, use, and interpret reliable sources. Case studies also require and build group learning skills and allow integration across many different disciplines and systems. For example, anatomy can be investigated along with histology and physiology to gain perspective on many disease processes. Many studies have indicated the use of case studies increased retention. As an undergraduate at Colorado State University I have seen an opportunity for case based learning in many science classes. I have created a workbook of case studies for Neuroanatomy students including answer keys to facilitate problem-based learning.
EXPERTISE: Education, Human Anatomy, Neuroanatomy