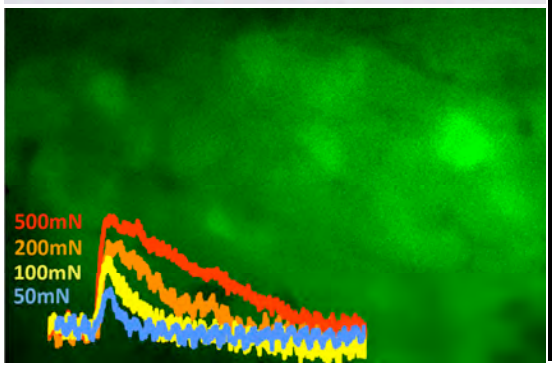
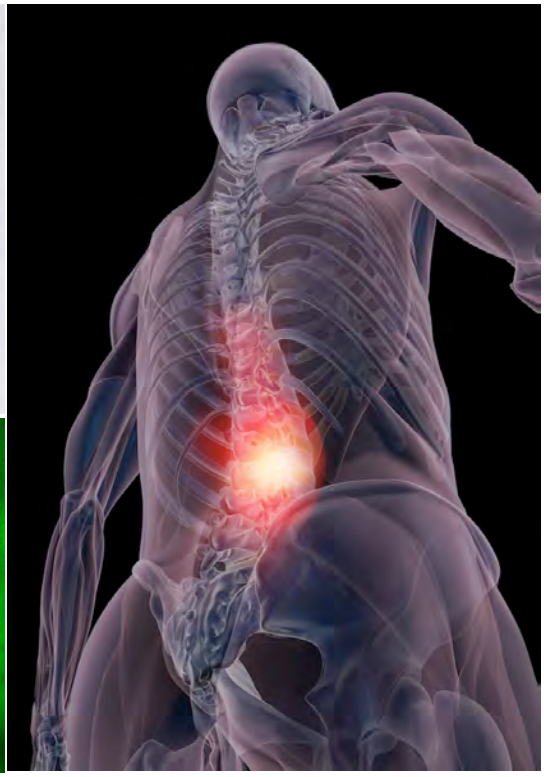
12TH ANNUAL MEETING OF THE

FRONT RANGE NEUROSCIENCE GROUP

December 10, 2014
Hilton Fort Collins





FRONT RANGE NEUROSCIENCE GROUP

12th Annual Meeting: December 10, 2014
Hilton Fort Collins
Registration 10am; Program 10:30am – 6:30pm

10:30-11:30 – Data Blitz

"New in the Front Range"

Noon-3pm – Lunch, Posters, Vendors!
12:30--1:45 ODD
1:45--3:00 EVEN

3-4pm – Award Winning Student presentations
Kristen Smith (Univ of Wyoming)
Peter Grace (Univ Colorado Boulder)
Maneesh Kumar (CU Anschutz)

4:00 - 4:30pm – Coffee break, stretch

4:30-5:30pm –Keynote: "Deciphering Neuronal Circuits Mediating Anorexia and Pain"

Richard Palmiter, PhD

Professor, Department of Biochemistry; Investigator, HHMI
University of Washington
http://www.hhmi.org/research/genetics-mouse-behavior

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

SfN http://FRNG.colostate.edu SfN

Morning Session: Data Blitzing through “new" in the Front Range

Alysia Vrailas-Mortimer, Assistant Professor, Dept. of Biological Sciences, University of Denver: "Aging and neurodegeneration in Drosophila”

Erik Oleson, Assistant Professor, Dept. of Psychology, University of Colorado-Denver: “Accumbal phasic dopamine release events shape operant behavior through computation of conditioned stimuli"

Jared Bushman, Assistant Professor, School of Pharmacy, University of Wyoming: “Astroglyconeuroengineering”

Ashley N. Fricks-Gleason, Assistant Professor, Dept. of Psychology & Neuroscience, Regis University: “The effect of exercise on the neurochemical consequences of methamphetamine abuse”

Nidia Quillinan, Assistant Professor, Dept. of Anesthesiology, University of Colorado-Anschutz Medical Campus: “Purkinje cell loss and cerebellar deficits resulting from global cerebral ischemia”

Yumei Feng, Assistant Professor, Dept. of Biomedical Sciences, Colorado State University: “New paradigm in the renin-angiotensin system and neurogenic hypertension”

Donald Rojas, Associate Professor, Dept. of Psychology, Colorado State University: “TBD”

Emily Bates, Professor, Dept. of Pediatrics, University of Colorado-Anschutz Medical Campus: “Ion channel activity regulates morphogen release”

Michael Saddoris, Assistant Professor, University of Colorado-Boulder, Dept. of Psychology and Neuroscience: "Causally linking phasic dopamine release in the nucleus accumbens to value-based decision making"

Lisa M. Wolfe, Research Scientist, Proteomics & Metabolomics Facility, Colorado State University: “Biological Mass Spectrometry at CSU – Supporting and Accelerating Scientific Research”

Acknowledgements:

Cover Page: Designed by Christina Dennison
Scientific images provided *by Kristen Smith (Univ of Wyoming), Peter Grace (Univ Colorado Boulder), and Maneesh Kumar (CU Anschutz).* 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: DSM Nutritional Products, Olympus America, and Sigma Aldrich. 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 Laura Joy 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 Christina Dennison, Ryan Tooker, and Mallory Shields from CSU, Dori R. Pitynski and Colleen M. Cassidy from Univ Wyoming, John Soltys and Anna Garske from UC Anschutz Health Science Campus, Casey O'Neil and Ryan Newsom from UC-Boulder and Stephanie Stout from DU. And additionally to Erin Bisenius, Brett Beal, and Sara Neys and the first year MCIN students for helping with attendee registration.

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, Kim Hoke, Qian-Quan Sun, Serge Campeau, Alysia Mortimer, 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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ORAL ABSTRACT SESSION

1. **Using GCaMP to measure inflammation-induced alterations in mechanical sensitivity of cutaneous primary afferents in vivo.**
   Kristen M. Smith[1], J DeBerry[2], BM Davis[2], CJ Woodbury[1]. From the [1]Department of Zoology and Physiology, University of Wyoming, Laramie, WY; [2]Medical University of Pittsburgh, Pittsburgh, PA.

2. **Therapeutic morphine prolongs neuropathic pain in rats: a role for TLR4 and inflammasome signaling in the lumbar spinal cord.**
   Peter M. Grace[1], Keith A. Strand[1], Erika L. Galer[1], Yingning Zhang[1], Debra Berkelhammer[1], Lisa I. Greene[1], Kenner C. Rice[2], Steven F. Maier[1], Linda R. Watkins[1]. From the [1]Department of Psychology and the Center for Neuroscience, University of Colorado Boulder, Boulder, CO; [2]Chemical Biology Research Branch, National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism, Rockville, MD.

3. **Decreased Glycolysis and Mitochondrial Respiration in a Zebrafish Model of Dravet Syndrome.**
   Maneesh G. Kumar[1], Shane Rowley[1], Ruth Fulton[1], Matthew T. Dinday[2], Scott C. Baraban[2], and Manisha Patel[1]. [1]Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO; [2]Department of Neurological Surgery, University of California San Francisco, San Francisco, CA.

ORAL ABSTRACTS

Kristen M. Smith, J DeBerry, BM Davis, CJ Woodbury.

Using GCaMP to measure inflammation-induced alterations in mechanical sensitivity of cutaneous primary afferents in vivo.

Inflammation causes the release of a variety of chemical mediators that can directly sensitize primary afferent neurons to subsequent mechanical, thermal, and/or chemical stimuli. Previous studies based on single cell, electrophysiological approaches have been limited in their ability to assess the temporal development of sensitization within a population of neurons. The use of genetically-encoded calcium indicators, such as GCaMP, provides researchers the unique ability to simultaneously monitor the activity of a population of cells in real-time. GCaMP allows non-invasive, quantitative analyses of neural activity, reliably measures neural responses to repeated stimuli over time, and is an ideal approach to detect change in primary afferent mechanical sensitivity in vivo. To determine the effects of a combination of inflammatory mediators, cutaneous primary afferent GCaMP responses to mechanical stimulation were optically recorded in vivo before and after a subcutaneous injection of “inflammatory soup” (IS), containing bradykinin, serotonin, histamine and prostaglandin E2 (10^{-5} M, 6.0 pH, 7mM K^{+}). For these studies, transgenic mice constitutively expressing GCaMP3 in all cells were anesthetized, artificially ventilated, decorticated, and the L6 dorsal root ganglion was exposed. Brief trains of electrical stimuli were used to locate innervation areas that corresponded with visual fields containing GCaMP-responding cells. The innervation area was glued onto the platform of a mechanical stimulator, and mechanical responses of cells within the visual field were obtained under baseline conditions (50-500mN, 1mm^2 probe). IS was then administered to the

innervation area (20µl, s.c.), while imaging neuronal responses. After waiting thirty minutes, mechanical stimulation was repeated and responses were imaged. Responses of specific ROI's were measured (ΔF/F) before and after IS using ImageJ. GCaMP signals were elicited in response to both mechanical (n=26) and chemical (n=22) stimulation. Some mechanosensitive neurons also displayed heat (7/26) and chemosensitivity (8/26). IS injection either significantly decreased (n=9; p=0.002), had no effect on (n=5; p=0.380), or significantly increased (n=6; p=0.001) the mechanical responses of primary afferents, compared to vehicle injection. Additionally, IS induced de novo mechanical sensitivity in cells that previously did not respond to mechanical stimulation (n=6). Furthermore, the seven heat-sensitive afferents all underwent mechanical sensitization after IS injection, either by exhibiting increased mechanical responses (n=3) or de novo mechanical sensitivity (n=4). These results suggest that IS-induced sensitization may be selective for a specific population of primary afferents. Future in vivo GCaMP studies may lead to the identification of specific subtypes of primary afferent fibers and key inflammatory agents that are involved in inflammation-induced hypersensitivity.

Peter M. Grace, Keith A. Strand, Erika L. Galer, Yingning Zhang, Debra Berkelhammer, Lisa I. Greene, Kenner C. Rice, Steven F. Maier, Linda R. Watkins.

Therapeutic morphine prolongs neuropathic pain in rats: a role for TLR4 and inflammasome signaling in the lumbar spinal cord.

Recent clinical studies, as well as rodent studies in our lab, revealed a previously unsuspected deleterious effect of treating pain with opioids. Despite their status as gold-standard therapeutic analgesics for neuropathic pain, opioids initiate microgliosis and pronociceptive cytokine release via Toll Like Receptor 4 (TLR4) in naïve animals. However, the behavioral and molecular impact of opioid-induced gliosis has not been defined in the presence of peripheral nerve injury, which also induces lumbar spinal gliosis per se. To address this clinically relevant question, we hypothesized that sciatic chronic constriction injury (CCI)-allodynia would be enhanced by subsequent repeated morphine in male F344 rats, involving TLR4, P2X7 receptor (P2X7R) and caspase-1, facilitating release of interleukin (IL)-1β. Beginning 10 days after CCI, morphine (5 mg/kg b.i.d.) or equivolume saline was administered for 5 days. Compared to vehicle, morphine significantly prolonged the duration of CCI-induced allodynia (n=6/group; $p<0.05$). Morphine also significantly elevated P2X7R, NFkappaB, NLRP3 and caspase-1 protein levels ($p<0.05$), and TLR4 and IL-1beta mRNA ($p<0.05$), in the ipsilateral lumbar dorsal quadrant (iLDQ), 5 weeks after dosing conclusion. Supporting a causal role for NLRP3 inflammasome activation in morphine-prolonged CCI-allodynia, continuous intrathecal infusion of inhibitors of TLR4 ([+]-naloxone; 60 µg/h), P2X7R (A438079; 30 ng/h), or caspase-1 (ac-YVAD-cmk; 1 µg/h) prevented prolonged allodynia when administered concomitantly with morphine, and abolished established morphine-prolonged CCI-allodynia when administered 5 weeks after morphine dosing (n=6/group; $p<0.05$). A single intrathecal IL-1 receptor antagonist dose (100 µg) also attenuated morphine-prolonged CCI-allodynia (n=6/group; $p<0.05$). In keeping with known pro-nociceptive roles for IL-1β, phosphorylation of the NR1 NMDA subunit was elevated, while GRK2 and GLT-1 levels were decreased in iLDQ 5 weeks after dosing conclusion ($p<0.05$). [+]-Naloxone coadministration during morphine treatment was sufficient to normalize P2X7R, NFkappaB, NLRP3, caspase-1, NR1, GRK2 and GLT-1 protein levels ($p<0.05$), and TLR4 and IL-1beta mRNA ($p<0.05$) in the iLDQ, 5 weeks after dosing. Finally, to determine the role of sustained microgliosis in maintaining morphine-prolonged CCI-allodynia, CD68-expressing cells (microglia) were transfected with an inhibitory Designer Receptor Exclusively Activated by a Designer Drug (DREADD). Intrathecal administration of the selective DREADD ligand, clozapine-n-oxide, attenuated morphine-prolonged CCI-allodynia (<0.05). These data suggest that morphine and the products of nerve injury interact, resulting in prolonged neuropathic pain via sustained inflammasome signaling. These data present an opportunity to pharmacologically

inhibit paradoxical pain enhancement while retaining the analgesic properties of morphine, as each is mediated via different receptors and underlying mechanisms.

Maneesh G. Kumar, Shane Rowley, Ruth Fulton, Matthew T. Dinday, Scott C. Baraban, and Manisha Patel.

Decreased Glycolysis and Mitochondrial Respiration in a Zebrafish Model of Dravet Syndrome.

Interictal hypometabolism is an important feature of many epileptic syndromes but has not been reported in Dravet syndrome (DS), a catastrophic childhood epilepsy caused by loss-of-function mutations in a voltage-activated sodium channel, Nav1.1 (SCN1A). Seizures in these children are often pharmacoresistant but can, in some cases, respond favorably to metabolic therapies such as the ketogenic diet. This raises the interesting possibility that metabolic dysfunction may occur in DS and contribute to its underlying pathophysiology. To address this, we developed novel methodology to assess real-time changes in bioenergetics in zebrafish larvae between 4 and 6 days post fertilization (dpf). Baseline and 4-aminopyridine (4-AP) stimulated glycolytic flux and mitochondrial respiration were simultaneously assessed using a Seahorse Biosciences extracellular flux analyzer. Scn1Lab mutant zebrafish showed a 1.7-fold decreased baseline glycolytic rates and a 1.5-fold lower baseline oxygen consumption rates (OCR) compared to controls. Increasing neuronal excitability with 4-AP resulted in an immediate increase in glycolytic rates in wild-type zebrafish; whereas mitochondrial OCR increased slightly and quickly recovered to baseline values. In contrast, scn1Lab mutant zebrafish showed a significantly slower and exaggerated increase of both glycolytic rates and OCR after 4-AP stimulation. A glucose metabolism PCR array identified five genes that were down-regulated in scn1Lab mutant zebrafish. Although the current theory is that relief of inhibition by sodium channel mutation on interneurons is the prevailing theory in DS leading to hyperexcitability, these results suggest metabolic dysfunction could also have a role. Baseline glucose and mitochondrial hypometabolism and delayed, but exaggerated, metabolic response to increased neuronal excitation in scn1Lab mutant zebrafish may shed light on mechanisms underlying disease pathophysiology in DS.

POSTER PRESENTATIONS

Cognition and Behavior

1) Aggressive behavior and medial prefrontal cortex activation during an escapable social encounter is altered by adolescent social isolation and is dependent upon the social history of the stimulus rat**.** Dayton J. Goodell, Megan Ahern, Jessica Baynard, Shawn Orcutt, Sondra T. Bland.

2) Exercise reward is independent of exercise controllability and involves the nigrostriatal dopamine pathway. Courtney A. Bouchet, Jonathan J. Herrera, Tyler Wieman, Parsa R. Ghasem, Jennifer Burns, Monika Fleshner, Benjamin N. Greenwood.

3) FMRI Investigation of the Accumulation of Probabilistic Categorical Information. Kurt Braunlich, CA Seger.

4) A Simple Classification Routine for Event-Related Brain-Computer Interfaces. Brittany K. Cabral, C Breeding, EM Forney, CW Anderson, PL Davies, WJ Gavin.

5) Voluntary exercise prevents reductions of mTOR mRNA in the prefrontal cortex and hippocampus following exposure to uncontrollable stress. Jennifer C. Burns, Courtney A. Bouchet, Parsa R. Ghasem, Peter J. Clark, Jonathan J. Herrera. Erica A. Sisneros, Agnieszka Mika, Monika Fleshner, Benjamin N. Greenwood.

6) Sensory Registration among Children with High-Functioning Autism Spectrum Disorders. Jewel Crasta, PL Davies, and WJ Gavin.

7) Cues predictive of rewards high in fat gain prominence over time. Rebecca A. Darling, Paige M. Dingess, Erin M. Smith, Travis E. Brown.

8) Exposure to a high-fat diet attenuates the locomotor-stimulating effects of cocaine. Paige M. Dingess, Brandon J. Anderson, Rebecca A. Darling, E. Kurt Dolence, Bruce W. Culver, and Travis E. Brown.

9) Stress-induced activity in the lateral habenula-dorsal raphe pathway is not sensitive to the dimension of behavioral control. Samuel D Dolzani, MV Baratta, LR Watkins, SF Maier.

10) Neurocognitive and Quality of Life Outcomes in Adult Survivors of Childhood Osteosarcoma. Michelle N. Edelmann, V Daryani, M Bishop, W Liu, C Stewart, KK Ness, D Mulrooney, TM Brinkman, C Kimberg, J Ehrentraut, DK Srivastava, LL Robison, MM Hudson, KR Krul.

11) The novel endocannabinoid drug MJN110 produces analgesia devoid from other typical cannabimetic effects. Melissa K. Fencl and Erik B. Oleson.

12) CEBL3: A Modular Platform for EEG Signal Analysis and Real-Time Brain-Computer Interfaces. Elliott M Forney, CW Anderson.

13) Cannabinoid receptor activation shifts temporally-engendered patterns of dopamine release. Jacqueline A Gallegos, Roger Cachope, Joseph F Cheer, Erik B Oleson.

14) The effect of heightened serotonin on aggression and mate choice in a sexually dimorphic species, Teleopsis dalmanni. Jaime L. Grace, AN Bubak, MJ Watt, KJ Renner, JG Swallow.

15) The novel endocannabinoid degradation inhibitor, MJN110, dose-dependently affects social behavior in rats. Raleigh Jonscher, A Alessi, EE Boxer, E Loetz, S Bland.

16) Dopamine Synthesis in the Ventral Tegmental Area in Rams with High or Low Libido. Avery C. Kramer, BS, Kathleen J. Austin, MS, Brenda M. Alexander.

17) Developmental changes in response monitoring ability from age 7 to 25 years with Woody filter technique: An event-related potential study. Mei-Heng Lin, Patricia L. Davies, William J. Gavin.

18) The Effect of Nicotine Administration and Withdrawal on Sleep in Mice. Hunter L. Mathews, Vivian Grimshaw, Alex Hatoum, Jerry Stitzel.

19) Behavioral Characterization of System xc- Mice. Elizabeth A. McCullagh, David E Featherstone.

20) Acute Moderate-to-Vigorous Physical Activity Results in Specific Executive Function Gains in Preschoolers. Lisa Schlueter McFadyen-Ketchum, SE Watamura.

21) Long-Term Cognitive Impairment from Early Age of Onset and Heavy Marijuana Use. Courtnie J. Paschall, Dr. Joseph Orr.

22) Increased phasic activation of mesolimbic dopamine neurons promotes negative reinforcement. Noah A. Rauscher, Jacqueline Gallegos, Erik B. Oleson.

23) Sexual orientation is associated with temporal changes in neural processing: An ERP study of in-group bias. Robert L. Ross, Maia T. Nguyen, Stephanie Bastidas, Lucy J. Troup.

24) In vivo optogenetic manipulation of dopamine neurons during cue motivated behavior. Scott Schelp, Jeremy Gage, Greg Krzystynaik, Erik B. Oleson.

25) Effects of Stressor Controllability on Prefrontal-Striatal Circuit Activity. George D. Trahan, MV Baratta, SD Dolzani, JS Tyler, LR Watkins, SF Maier.

26) Single-trial Classification of Error Related Negativity ERN. Fereydoon Vafaei, Chuck Anderson.

Development

27) Ion channels in development. Giri Dahal, Sarala Pradhan, Colleen Bartman, Amir Ahmed, Emily Bates.

28) c-Myb and its role in neural development. Coral J. Cabrera Montalvo, J Vu, S Vo, JA Stitzel.

29) Amino Acid Transmitter Phenotype of Proopiomelanocortin Neurons in the Arcuate Nucleus of the Hypothalamus During Postnatal Development. Christina S. Dennison and ST Hentges.

30) Estrogen dependent cell movements in different regions of the developing hypothalamus. Chad Eitel, Luke Schwerdtfeger, Stuart Tobet.

31) VIP neurons in the suprachiasmatic nucleus of neonatal mice with disrupted fibroblast growth factor signaling. Annie V. Miller, Scott I. Kavanaugh, Pei-San Tsai.

32) Cellular prion protein promotes olfactory sensory neuron development. Lindsay E Parrie, Jenna AE Crowell, Richard A Bessen.

Disorders of the Nervous System

33) Elevated neonatal iron intake potentiates progression in the R6/2 mouse model of Huntinton's disease. Kiersten L. Berggren, Jianfang Chen, Jonathan Miller, & Jonathan H. Fox.

34) Small Molecule Mimetics of Poly(sialic acid) for Nervous System Regeneration. Jared Bushman, G Loers, M Ezra, A Wallqvist, M Schachner.

35) Comparison of phospho-tau and cofilin-actin rod pathologies in the brains of subjects from a longitudinal aging study. Adlei Carlson, Laurie S Minamide, Stephen W. Scheff, Yi Zhou, Tristan Krug, and James R. Bamburg.

36) Exploring the role of prion protein expression level and profile on pathogenesis of chronic wasting disease. Jeffrey R. Christiansen, Sehun Kim, Glenn C. Telling.

37) Brain indoleamine-2,3-dioxygenase enzymatic activity is increased in the N171-82Q mouse model of Huntington's disease. David Donley, Andrew Olson, Merl Raisbeck, Jason Gigley, Jonathan Fox.

38) 1,1-bis(3'indolyl)-1-(p-chlorophenyl)methane (C-DIM12) provides dopaminergic phenotypic stability through Nurr1 interactions in neuronal cultures. Sean Hammond, Stephen Safe, and Ronald Tjalkens.

39) Thioltransferases TXN1 and TXNDC10 protect against neuronal atrophy in a lentiviral mouse model of Huntington's disease. Zhen Lu, Lorraine Barrows, Jianfang Chen, Jenna Moline, Jonathan Fox.

40) HSPB8: A candidate susceptibility factor in prion diseases. Julie A. Moreno, Vadim Khaychuk, Jifeng Bian, Carla Calvi and Glenn C. Telling.

41) The effects of exercise on methamphetamine-induced serotonin, tyrosine hydroxylase, and dopamine neurotoxicity in Rattus norvegicus. Monica Murray, Zoe Vlastos, Katherine Varley, and Ashley Fricks-Gleason.

42) Indoleamine-2,3-dioxygenase Activity is Modulated by Iron Status: implications for neurodegenerative diseases. Andrew Olson, David Donley, Merl Raisbeck, Jason Gigley, Jonathan Fox.

43) Alphavirus-manganese interactions and dopaminergic neurodegeneration. Aaron T. Phillips, KE Olson, TA Aboellail, RJ Smeyne, RB Tjalkens.

44) Activation of the nuclear receptor Nur77 by a novel diindolylmethane analog suppresses inflammatory gene expression in primary astrocytes. Katriana A Popichak, RB Tjalkens, S Safe.

45) Drosophila p38 MAP Kinase Regulates Age-dependent Protein Homeostasis. Sarah Ryan, Amelia M. Burch, Subhabrata Sanyal, and Alysia Vrailas-Mortimer.

46) Emerging Roles of Synaptotagmin: Modeling Neurogenic Disorders in Drosophila. Mallory Shields, Matthew Bowers, Roger Whittaker, Rita Horvath, Noreen Reist.

47) PrPC-dependent cofilin-actin rod formation occurs through neurite-specific activation of NADPH oxidase. Keifer P. Walsh, Laurie S. Minamide, Alisa E. Shaw, Adlei Carlson, Lindsey Whittington, Michael Ruff, Mark D. Zabel, Thomas B. Kuhn, James R. Bamburg.

Neural Excitability, Synapse, and Glia

48) Mitochondria mediate local stimulation of L-type calcium channels in cerebral artery smooth muscle. Nathan L. Chaplin, GC Amberg.

49) Fasting increases agouti-related peptide neuron inhibition of proopiomelanocortin neurons in the hypothalamus. Matthew S. Dicken, AR Hughes, ST Hentges.

50) A novel role of adenosine A2B receptors in regulating calcium-activated small conductance potassium (SK) channels at the synapse. Anna K Garske, LB Weitzel, RJ Traystman, PS Herson.

51) Linking Aβ42-Induced Hyperexcitability to Neurodegeneration, Learning and Motor Deficits, and a Shorter Lifespan in an Alzheimer's Model. Y Ping**,** Hahm Eu-Teum, G Waro, Q Song, D Vo-Ba, A Licursi, H Bao, L Ganoe, K Finch, and S Tsunoda.

52) Exploring the role of caudal brainstem proopiomelanocortin neurons in feeding circuits. Alexander R. Hughes, MS Dicken, ST Hengtes.

53) Layer Dependent Dopaminergic D1 receptor activation effects on frequency dependent short-term synaptic plasticity. Jonna M. Leyrer-Jackson, Mark P. Thomas.

54) Resistance or susceptibility to desensitization by presynaptic mu opioid and GABAB receptors is not due to differential effector coupling. Reagan L. Pennock, Shane T. Hentges.

55) Regulation of Synaptic Plasticity by Neuronal Lactic Acid Transport and Metabolism. Amy L. Uhernik, R Cerda, Jeff P. Smith.

56) Mechanisms of Serotonin-2A Mediated Recurrent Oscillatory Bursting in Layer 5 Pyramidal Neurons of the mPFC. Michael S. Spindle, Mark P. Thomas.

Neuroendocrine

57) Reproductive phenotype and GnRH system in Fgfr3-deficient mice. Samantha J. Bonelli, SI Kavanaugh, AN Le, FC Doherty, PS Tsai.

58) Acute stress selectively and rapidly induces per1 expression in the medial prefrontal cortex, suprachiasmatic nucleus, and paraventricular nucleus of male and female rats. Lauren E. Chun, Sarah Morton, Liz Woodruff, Laura R. Hinds, & Robert L. Spencer.

59) Reactive Oxygen Species Modulate Local L-type Calcium Channel Signaling in Gonadotropes. An K. Dang, Dilyara Murtazina, Christianne Magee, Amy M. Navratil, Colin M. Clay, Gregory C. Amberg.

60) Dynamin is required for GnRH signaling to L-type calcium channels and activation of ERK. Brian S. Edwards, AK Dang, GC Amberg, CM Clay, AM Navratil.

61) Inhibition of organic cation transporter 3 (OCT3) in the central nucleus of the amygdala increases extracellular serotonin and reduces fear expression. James E. Hassell, Jr., H Li, J Rogers, S Ferrell, M Orchinik, CA Lowry, KJ Renner.

62) Functional evolution of gonadotropin-releasing hormone and adipokinetic hormone: Studies from the sea hare, Aplysia californica. Joshua I Johnson, PS Tsai.

63) Gonadotropin releasing hormone stimulates histone citrullination in gonadotrope cells. Shaihla A. Khan, Coleman H. Young, Brian D. Cherrington, Amy M. Navratil.

64) In a Salted State: High Salt Affects Metabolism and Puberty. Dori R. Pitynski, FW Flynn, DC Skinner.

65) Salt and Metabolism: A Dietary Investigation of Respiration and Lipid Output. Margaret Schmill, Dori Pitynski, Micah Ross, Donal Skinner.

66) Fetal Programming of Newborn Leukocyte Telomere Length. Stephanie A. Stout, Judith E. Carroll, Laura M. Glynn, Deborah A. Wing, Elysia Poggi Davis.

67) Glucocorticoid-dependent diurnal modulation of conditioned fear extinction and recall. Liz R Woodruff, Benjamin Greenwood, Lauren E Chun, Sara Fardi, Laura R Hinds, Robert L Spencer.

Neuroscience Outreach/Education

68) Muscles Alive! A novel, experiential neuroscience education outreach program for elementary, secondary, and university students. Breonna E. Bost, T. Evan West, Katelyn E. Timroth, and Brian L. Tracy.

Sensory and Motor Systems

69) Characterization of rubro-cerebellar reciprocal circuits. Christy S. Beitzel, Brenda D. Houck, Abigail L. Person.

70) Characterization of AgRP Immunoreactivity in Taste Buds of C57 and ob/ob Mice. Derek George, Leslie M. Stone.

71) Development and Testing of an Electrotactile Tongue Stimulation Device for Sensory Substitution. Joel A. Moritz Jr, John D. Williams, Leslie M. Stone-Roy.

72) Cellular and population analyses of signal filtering at olfactory bulb glomeruli. Joseph D. Zak, Nathan E. Schoppa.

Translational Neuroscience

73) Dietary queen bee acid reduces anxiety and stress-related weight loss in aged male rats. Michael J. Weiser, Kelly M. Wynalda, M. Hasan Mohajeri, N. Salem Jr., and Christopher M. Butt.

74) Dietary N-methylserotonin from Japanese pepper (Zanthoxylum piperitum) regulates skin temperature in a female rat model of menopause-related hot flash. Michael J. Weiser, M. Hasan Mohajeri, and Christopher M. Butt.

Art

75) Elliot Forney, Colorado State University. “Energy” Electroencephalography (EEG) is a technique for measuring electrical activity generated by the brain using an array of electrodes placed on the surface of the scalp. This print is a modified spectrogram showing the energy contained in an EEG signal localized across both time and frequency using a continuous wavelet transform.

76) Devan Kallas, Colorado State University. Untitled.

ABSTRACTS

Cognition and Behavior

1) Aggressive behavior and medial prefrontal cortex activation during an escapable social encounter is altered by adolescent social isolation and is dependent upon the social history of the stimulus rat

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Adolescent social isolation (isolation rearing) has been shown to increase aggressive behavior and alter medial prefrontal cortex (mPFC) function in social species such as rats. Here we exposed male rats to 4 weeks of postweaning social isolation (ISO) or group (GRP) housing, then exposed them to an apparatus that allowed an escapable social interaction with a stimulus rat that was either ISO or GRP housed. Rats were exposed to 3 trials in the apparatus, with either all 3 of the trials or the last trial with a stimulus rat, and brains were harvested 100 min after the last trial for c-fos and ARC immunohistochemistry. ISO rats spent less time in the escape chamber than GRP rats. Analysis of social behaviors indicated that ISO rats spent more time engaged in social interaction, aggressive grooming, and boxing than did GRP rats. Interestingly, all rats engaged in more social interaction and bouts of boxing with ISO stimulus rats. Subregion- and stimulus-dependent changes in c-fos and ARC expression were observed. Rats exposed to GRP stimulus rats on the third trial had greater c-fos and ARC expression in the prelimbic region of the mPFC than those exposed to an ISO stimulus rat on the third trial. Isolation rearing increases aggression during escapable social encounters, and the rearing condition of the stimulus rat in a social encounter is an important component of behavioral and neural outcomes for both isolation and group-reared rats.

2) Exercise reward is independent of exercise controllability and involves the nigrostriatal dopamine pathway

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Dopamine (DA) reward circuits are implicated in stress-related disorders such as anxiety and depression. Exercise reduces the incidence of stress-related disorders, but the contribution of exercise reward to exercise-induced stress resistance is unknown. We have reported that the protective effects of exercise are independent of exercise controllability; whereby both voluntary and forced wheel running protect rats against behavioral consequences of stress. Voluntary exercise is a natural reward, but whether rats find forced wheel running rewarding is unknown. Moreover, the contribution of DA systems to exercise reward is not well characterized. The mesolimbic DA pathway is classically implicated in reward and is sensitive to voluntary exercise. Emerging data implicate the nigrostriatal DA pathway, traditionally associated with movement, in reward. The contribution of the nigrostriatal DA pathway to exercise reward has been difficult to determine due to its dual role in movement and reward. The goal of the current studies was to determine whether the rewarding effects of wheel running depend on its controllability and, if so, the neural pathways by which it elicits its rewarding effect. Male F344 rats were divided into voluntary and forced exercise groups. Rats in the forced group were placed in wheels that were rotated by motors on a predetermined schedule resembling the typical pattern of voluntary running. For 30 d, rats were moved into voluntary or forced wheels or, on alternating nights, an empty cage. Two hours after wheel or empty cage exposure, rats were placed on one distinct side of a conditioned place preference (CPP) chamber. One side was always paired with running (paired) and the opposite side was paired with the empty cage (unpaired). Results of probe tests conducted 10, 20 and 30 d after the start of CPP training indicated that both voluntary and forced wheel running are rewarding. Rats spent more time on the side of the CPP chamber paired with exercise, regardless of controllability. After the final probe trial, and 24 hours after the last running exposure, rats were placed on either the paired or unpaired side and sacrificed 30 min later. Double fluorescent in situ hybridization (cfos / TH in the midbrain regions and cfos / dynorphin in the dorsal and ventral striatum) revealed that re-exposure to the paired side elicited conditioned activation of both the mesolimbic and nigrostriatal DA pathways independent of differences in locomotor activity. In a separate experiment, male F344 rats were assigned to sedentary, voluntary wheel (VW), or forced wheel (FW) running conditions. After 6 weeks of running or sedentary conditions, the animals were sacrificed and the brains were removed and stained for protein via immunohistochemistry. Preliminary data suggest that voluntary and forced wheel running act through similar DA pathways. These data suggest that voluntary and forced wheel running can be rewarding and these rewarding effects likely involve both the nigrostriatal and mesolimbic DA pathways. The rewarding effects of exercise could contribute to exercise-induced stress resistance. **Keywords:** Exercise, Reward, Dopamine

3) FMRI Investigation of the Accumulation of Probabilistic Categorical Information

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Our task required participants to categorize different “amoeba” based upon the probabilistic evidence provided by different stimulus features, which were presented, one-by-one, over four discrete steps. By independently controlling the instrumental contingencies for each feature, and by temporally-jittering the event onsets, we were able to build and compare several computational models of processes occurring during the deliberation, commitment and feedback epochs of each trial. Of note, our results provide evidence of two mechanisms subserving the flexible modulation of the speed-

accuracy trade-off: gain modulation of the striatum, and gain modulation of integrated effector-specific evidence. We also found that activity within different regions of the striatum tracked the temporal evolution of different decision-related variables. Activity within regions of the putamen reciprocally connected with the somatomotor network tracked effector-specific evidence (e.g., evidence towards a response with the left hand), while regions of the putamen associated with the ventral-attention network tracked the precision of the exogenous information (i.e., the strength of evidence for either response). **Keywords:** decision-making, fMRI, categorization, speed-accuracy trade-off

4) A Simple Classification Routine for Event-Related Brain-Computer Interfaces

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Brain-computer interfaces (BCI) establish a direct line of communication between an individual's brain and a computerized device using non-invasive electroencephalography (EEG). By simply altering one's mental state, an individual can control electronic devices making BCI ideal for individuals with severe motor impairments. One BCI application, the P300 speller, allows an individual to spell out words and sentences by focusing on a particular target letter. When the target letter appears, the individual will elicit a P300 response, which is a large, positive voltage deflection that occurs approximately 300ms after the onset of a stimulus. When the brain's response to the target is averaged across a number presentations, the P300 response to a target letter is larger than the P300 to a non-target letter. However, identifying the P300 in the brain's response to a single target presentation is often problematic due to noise in the brain signal (e.g., background brain processing unrelated to the task). A number of computer algorithms have been developed to detect the P300 and other features of the brain response in order to classify each stimulus presentation as a "target" or "non-target" with varying success. The goal of the present study was to determine whether a simple algorithm for feature selection could accurately classify the data from a simulated P300 speller routine. Sixteen participants completed the study. Nine participants were able-bodied individuals who completed the study in a lab setting, and 7 participants with severe motor impairments were tested in their homes. Letter characters were displayed one-at-a-time on a computer screen in front of the participant. Participants were asked to silently count the number of presentations of a particular target letter (p, b, or d) in three separate blocks. Each block consisted of a total of 80 trials, 20 of which were target letters. In the current study, data were analyzed offline for classification. The algorithm was developed in Matlab code using a template-matching approach. For each target letter and for each individual, two templates were created, target and non-target, by averaging the first 10 segments of "target" letters or "non-target" letters respectively. The remaining data were classified as "target" or "non-target" by determining which template each segment matched the most closely based on measures of variance and correlation. Early results show promise for this simple classification routine. **Keywords:** Brain-Computer Interface, ERP, EEG, P300, Classification

5) Voluntary exercise prevents reductions of mTOR mRNA in the prefrontal cortex and hippocampus following exposure to uncontrollable stress

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Previous research demonstrates benefits of exercise, including enhancing learning and memory and producing resistance against stress-related psychiatric disorders such as depression and anxiety. The mechanisms underlying these beneficial effects of exercise remain unknown. The mammalian target of rapamycin (mTOR) is implicated in biochemical reactions involving the regulation of cell growth, proliferation, and survival. mTOR has recently been implicated in enhancing learning and memory in the hippocampus. Previous research demonstrates stimulation of mTOR may be implicated in mediating antidepressant effects in the prefrontal cortex by increasing levels of synaptic proteins. In particular, it was shown that a low does of NMDA receptor antagonist ketamine induces mTOR signaling, and is followed by rapid biochemical and behavioral antidepressant effects. Inhibition of PFC mTOR with intra-PFC rapamycin eliminated the anti-depressant effects of ketamine. The effects of exercise on mTOR are unknown. As exercise both enhances learning and memory and produces protective effects against depression, mTOR therefore may be involved in the underlying mechanisms of both enhanced learning and memory and anti- depressant and anxiolytic effects. The present study sought to examine if exercise would increase levels of mTOR in the hippocampus and PFC of rats, and prevent stress-induced decreased of mTOR following exposure to uncontrollable tail shock. Rats were exposed to 6 weeks of either sedentary activity or voluntary wheel running. At 6 weeks, half of each group was exposed to no stress or uncontrollable tail shock. Immediately following exposure to stress, rats were sacrificed and levels of mTOR mRNA were measured using in situ hybridization. Exercise initially decreased levels of mTOR in the hippocampus. Conversely, levels of mTOR in the hippocampus increased following exposure to stress in ran rats. Stress appears to reduce levels of mTOR in sedentary rats. To further investigate these findings, a second experiment analyzed levels of phosphorylated mTOR in the PFC following 6 weeks of exposure to either sedentary activity, voluntary or forced wheel running, using immunohistochemistry. To determine the relative phosphorylation of mTOR between groups cells were grouped into light and medium/dark counts. While no significant difference was found in the total number of cells between groups, voluntary wheel runners displayed higher counts of light, medium and dark cells compared to the forced and sedentary groups. Both

forced and voluntary wheel runners showed greater expression of phosphorylated mTOR than sedentary rats. Thus, wheel running increased levels of phosphorylated mTOR regardless of exercise controllability.

6) Sensory Registration among Children with High-Functioning Autism Spectrum Disorders

Jewel Crasta1, PL Davies1, and WJ Gavin2. From the departments of 1Occupational Therapy, and 2Human Development & Family Studies Colorado State University, Fort Collins, CO.

This study sought to determine whether children with high-functioning autism spectrum disorders (ASD) differ from typically developing children on neurophysiological measures of auditory sensory processing. Fifteen children with ASD and 15 age- and gender-matched typically developing children, ages 5 to 12 years, participated in this study. Electroencephalography (EEG) recordings were made while participants watched a silent movie and heard random presentations of four auditory stimuli at two different frequencies (1 and 3 kHz) and at two different intensities (50 and 70 dB). Amplitude and latency measures were obtained for the N1, P2, N2, and P3 components from the averaged event-related potentials (ERPs) for each of the four auditory stimuli. An ANCOVA for the amplitude of P3 component, controlling for number of averaged segments revealed a significant main effect of group. Children with ASD demonstrated significantly smaller P3 amplitudes than typically developing children, suggesting that children with ASD have reduced cognitive processing to these auditory stimuli. Children with ASD also had significantly smaller N2 amplitudes for the low frequency soft tone stimuli compared to typically developing peers, suggesting increased difficulty in automatic stimuli discrimination. In conclusion, this study shows that children with ASD display different brain processing mechanisms to auditory sensory stimuli compared to typically developing children. These results can help practitioners understand the neurophysiological basis of behavioral manifestations of ASD, especially those behaviors that relate to sensory experiences in everyday activities. **Keywords:** ERP, Sensory processing, Autism spectrum disorders

7) Cues predictive of rewards high in fat gain prominence over time

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Sensory cues predictive of highly palatable food rewards have been shown to induce a motivational craving state even in the absence of an energy balance deficit, thus triggering maladaptive overeating behaviors. In rats, craving may be assessed by measuring their propensity to respond on a lever for a cue previously associated with a reward and craving has been shown to increase in intensity progressively over time for such rewards as cocaine and sucrose. The increased craving behavior manifests in rats as an enhancement in lever responding to contingent cues after extended abstinence periods and subsequently coined, incubation of craving. We hypothesized that cues paired with food pellets calorically high in fat would undergo an incubation of craving effect. To test this hypothesis our lab did a series of experiments to determine if exposure to high-fat (HF) pellets associated with a tone and light (compound cue) would cause an incubation of craving effect over time. In our experiments we assessed the behavioral response after 30 days of abstinence (CE30) from HF. Our results indicate that lever pressing for HF increases from one day of abstinence (CE1) to CE30 (35.0 ± 8.2 vs. 92.2 ± 13.5, $p<0.05$, n=10). We used sucrose pellets as a positive control and observed an increase in lever pressing for sucrose from CE1 to CE30 as well (39.2 ± 7.2 vs. 90.7 ± 11.0, $p<0.05$, n=10). However, cues paired with normal chow pellets do not show as robust of an incubation effect (29.3± 8.8 vs. 65.9 ± 10.0, $p<0.05$, n= 10). We conclude that exposure to cues paired with HF undergo a time-dependent increase in prominence, which may contribute to maladaptive food seeking behaviors. **Keywords:** incubation of craving, high-fat, self-administration

8) Exposure to a high-fat diet attenuates the locomotor-stimulating effects of cocaine

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According to the National Institute of Health over consumption of diets rich in high-fat (HF) is one of the contributing factors to the obesity epidemic. Various laboratories have shown that exposure to a HF diet evokes changes in dopaminergic signaling within the reward circuitry. However, the effect HF has on general reward processing is still unclear. In our first set of experiments, we examined the effects of HF diets on cocaine-induced locomotion to assess whether HF may influence the sensitivity to the stimulating effects of cocaine. Rats were placed on either a normal chow (NC), HF ad libitum, or HF calorically restricted diet for 1 or 3 weeks. Animals were then tested in locomotor boxes to assess activity via photobeam breaks. Cumulative activity induced by novelty, saline injection (intraperitoneal (i.p.)) or cocaine (5 or 15mg/kg, i.p.) was assessed for each dietary condition. At 1 week there was a significant gain in body weight but no significant differences in any of the dietary groups with regard to locomotor activity. However at 3 weeks, there was a significant increase in body weight and decrease in beam breaks in the HF group injected with 15 mg/kg cocaine (21,128 ± 1,721, n=14) compared to NC (33,705 ± 2,965, n=8). The same reduction in cocaine-induced (15mg/kg) locomotor activity was observed in the HF calorically restricted group (20,286 ± 2,623, n=14) when compared to normal chow controls (30,342 ± 3241, n=9). Our results indicate that the HF diet is having an effect on cocaine-induced locomotor activity and this effect is independent of body weight. To address whether there is a general hypo-responsiveness of the reward circuitry in animals exposed to a HF diet an additional set of rats were placed on ad libitum HF or NC diets for 3 weeks, fasted for a 24-hr period and subsequently tested in an overnight sucrose self-administration task. Our results show that rats fed a HF diet have a significant attenuation in lever responses (429 ± 50, n=6) compared

to the NC controls (858 ± 45, n=8). In our final experiment, animals were fed either a HF calorically restricted or NC ad libitum diet for 3 weeks. Following dietary exposure, extracellular basal dopamine levels were assessed using high-performance liquid chromatography (HPLC) in the prefrontal cortex (PFC), nucleus accumbens (NAc), ventral tegmental area (VTA), hippocampus, and amygdala. Our results demonstrate a significant increase in basal dopamine in the PFC in the HF group (979.487 ± 410.584, n=8) relative to NC controls (100.000 ± 40.176, n=8). Overall, our results lead us to conclude that exposure to the HF diet elicits changes in reward processing that manifests as a reduction in reward sensitivity. **Keywords:** high-fat, cocaine, DiI staining, spines

9) Stress-induced activity in the lateral habenula-dorsal raphe pathway is not sensitive to the dimension of behavioral control

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Previous research from our laboratory has shown that controllable, but not uncontrollable, stressors modulate the neurochemical and behavioral outcome of the stressor. During uncontrollable (inescapable) tail shock (IS), but not controllable (escapable) tail shock (ES), serotonergic (5-HT) neurons in the dorsal raphe nucleus (DRN) are activated. IS sensitizes the DRN and results in acute anxiety-like behavior in rats. Activation of the DRN is both necessary and sufficient for the consequences of IS. The lateral habenula (LHb) provides the primary glutamatergic input to the DRN and dysregulation of both the LHb and DRN is implicated in stress-related mood disorders. In the present study we examined the role of the LHb in regulating the behavioral response to acute stress. We first sought to determine whether the LHb is differentially activated by the controllability of the stressor. ES and IS resulted in an equivalent increase in Fos protein in the LHb, as compared to home cage controls (HC). Next, we measured Fos expression restricted to the LHb-to-DRN pathway. The retrograde tracer Fluorogold (FG) was injected into the DRN and Fos protein was quantified in FG-positive cells in the LHb following ES, IS or HC. ES and IS yielded a similar increase in activation of the LHb-to-DRN pathway, with no effect of controllability of the stressor. Finally, we implemented an optogenetic strategy to determine whether silencing the LHb during IS would protect against the behavioral consequences of IS. Halorhodopsin silencing of the LHb during IS produced resistance to the anxiety-like behavioral outcome of IS, as measured in a juvenile social investigation test. These data suggest that the LHb modulates DRN activity during stress, and that silencing this pathway during or following adverse events may protect against stress-related mood disorders. **Keywords:** stressor controllability, optogenetics

10) Neurocognitive and Quality of Life Outcomes in Adult Survivors of Childhood Osteosarcoma

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Purpose: To examine neurocognitive and quality of life outcomes in long-term survivors of childhood osteosarcoma treated with high dose intravenous (HDIV) methotrexate. Patients and Methods: Survivors of osteosarcoma (n=80; mean [SD] age = 38.6 [7.12] years; time since diagnosis = 24.7 [6.6] years; 42.3% female) were recruited from the St. Jude Lifetime Cohort Study and were compared to community controls (n=39; age = 39.03 [11.71] years; 56.4% female). Performance on neurocognitive testing was compared between groups and to national normative data. Within survivors, neurocognitive function was examined in relation to pharmacokinetic indices of methotrexate exposure and current chronic health conditions, which were assessed through medical examination and coded according to Common Terminology Criteria for Adverse Events v4.0 (CTCAE). Patient-reported outcomes were also collected. Results: Compared to community controls, survivors demonstrated lower reading (p=0.01), attention (p=0.002), memory (p=0.02), processing speed (p<0.001) and executive function (p=0.006). Survivors also fell below national norms on these measures, as well as on mathematics (p=0.01). Neurocognitive performance was not associated with cumulative HDIV methotrexate or anthracycline. Pharmacokinetic indices of methotrexate concentration, clearance and exposure were not associated with performance. Any grade 3 or 4 CTCAE cardiac, pulmonary or endocrine condition was associated with poorer memory (p=0.01) and slower processing speed (p=0.002). Survivor-reported poor general health was associated with lower mathematics (p=0.04), sustained attention (p=0.05) and processing speed (p=0.006). Conclusions: Long-term survivors of osteosarcoma are at risk for neurocognitive impairment, which, nearly 25 years post-diagnosis, is related to current chronic health conditions. **Keywords:** Childhood cancer, chemotherapy, methotrexate

11) The novel endocannabinoid drug MJN110 produces analgesia devoid from other typical cannabimetic effects

Melissa K. Fencl and Erik B. Oleson. From the Department of Psychology, University of Colorado Denver.

MJN110 will increase analgesia without producing a full CB1 agonist profile (i.e., hypomotility, catalepsy, and hypothermia). It is predicted that the experimental results and findings will provide evidence that MJN110 provides tangible and measurable therapeutic benefits to treat a variety of patients through pain alleviation without the negative side effects (e.g., hypomotility) associated with a full CB1 agonist. **Keywords:** Endocannabinoid, Tetrad, MJN110, Analgesia

12) CEBL3: A Modular Platform for EEG Signal Analysis and Real-Time Brain-Computer Interfaces
Elliott M Forney, CW Anderson. From the Department of Computer Science, Colorado State University, Fort Collins, CO.
Non-invasive Brain-Computer Interfaces (BCI) are emerging technologies that may have a number of potential applications. For instance, BCI may be useful for the development of assistive devices, predicting the onset of epileptic seizures, monitoring of the state of anesthesia, detecting changes in emotional state and augmenting communication channels in computer games and other environments. Unfortunately, however, the software tools currently available for analyzing Electroencephalography (EEG) signals do not possess all of the qualities that are desirable for the rapid development of novel BCI technologies. With current software tools it is either impossible or impractical to either (1) perform automated real-time analysis (2) implement sophisticated interactive user interfaces (3) extend the software with novel signal processing and pattern analysis algorithms. The Colorado Electroencephalography and Brain-Computer Interfaces Laboratory (CEBL) is a software platform for performing EEG signal analysis and real-time BCI experiments that is currently under development at the Colorado State University Brain-Computer Interfaces Laboratory. CEBL aims to address the shortcomings of current EEG analysis tools in several ways. First, CEBL is written in the Python programming language and has a modular architecture. This allows each component to be extended or modified as a researcher sees fit. Second, CEBL follows a two-tiered design approach. The first tier contains general routines for signal analysis, EEG management and visualization as well as various machine learning algorithms. This makes the first tier suitable for offline EEG analysis and testing experimental methods. The second tier contains all of the components necessary for real-time processing, including EEG acquisition, stream buffering, animated visualization, widgets and various user interface components. The CEBL design allows BCI researchers to streamline the development of new BCI by first testing experimental methods in an offline fashion followed by the creation of interactive prototypes that leverage existing components. In the present work, we describe the CEBL design principals and discuss how the shortcomings of other EEG analysis tools and BCI frameworks are addressed. We also introduce several prototype BCI modules written for the CEBL platform. **Keywords:** Electroencephalography, Brain-Computer Interfaces

13) Cannabinoid receptor activation shifts temporally-engendered patterns of dopamine release
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Cannabinoids are thought to disrupt temporally controlled behaviors, possibly by increasing dopamine (DA) concentrations in the mesocorticolimbic system. Timing can be assessed using fixed-interval (FI) schedules, which reinforce behavior on the basis of time. It remains unknown how cannabinoids modulate DA release when responding under FI conditions, or how subsecond DA release is related to time in these tasks. Here, we hypothesized that cannabinoids would accelerate timing behavior in an FI task while also augmenting a temporally relevant pattern of DA release. To assess this, we measured DA concentrations in the nucleus accumbens using fast-scan cyclic voltammetry while mice responded for food under the influence of cannabinoids in an FI task. Our data reveal that DA concentrations decrease proportionally to interval duration, suggesting that DA encodes time in FI tasks. Furthermore, the cannabinoid receptor agonist WIN 55 212-2 dose-dependently increased DA release and accelerated timing behavior in a CB1 receptor-dependent manner, suggesting that cannabinoid receptor activation can modify timing behavior by enhancing time-engendered patterns of s release. Additionally, we uncovered a specific role for endogenous cannabinoid tone, as elevations in only one of the two well characterized endocannabinoids (i.e. 2-arachidonoylglycerol) increased the temporal response pattern similar to WIN 55 212-2. **Keywords:** dopamine, cannabinoids, endocannabinoids

14) The effect of heightened serotonin on aggression and mate choice in a sexually dimorphic species, Teleopsis dalmanni
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Sexually dimorphic species of stalk-eyed flies are known to experience intense sexual selection, both intersexual and intrasexual. Males with longer eyestalks are both more attractive to females and more likely to win contests with other males. Serotonin (5-HT) is known to play a role in aggressive behavior in this species, and males with artificially elevated 5-HT levels are more likely to win competitions with size-matched opponents over a food source. However, it is unknown whether the aggressive tendencies favored in intrasexual competition are also favored in intersexual mate choice. Furthermore, the increase in aggression in response to elevated 5-HT levels may be sexually dimorphic, and it is unknown whether females also increase aggression when 5-HT levels are raised. We first examined whether females treated with 5-HTP (5-hydroxytryptophan, the serotonin precursor that is converted to serotonin in the brain) are more likely to exhibit aggressive behaviors towards other females. We then tested whether males treated with 5-HTP are preferred in mate choice trials. These studies provide insight into potential dimorphism in response to elevated serotonin and potential conflict over optimal serotonin levels for intra- vs. intersexual selection.

15) The novel endocannabinoid degradation inhibitor, MJN110, dose-dependently affects social behavior in rats
Raleigh Jonscher1,2, A Alessi2, EE Boxer1,2, E Loetz2, S Bland2. From the 1Department of Integrated Biology, University of Colorado Denver; 2Department of Psychology, University of Colorado Denver.

Endocannabinoids (eCBs) are a class of bioactive lipids that act as retrograde neuromodulators and have been implicated in a wide variety of behavioral processes, including the regulation of anxiety and aggression. The two principal eCBs are anandamide (AEA) and 2-arachidonoylglycerol (2-AG); these are catabolized by the enzymes Fatty acid amide hydrolase (FAAH) and Monoacylglycerol lipase (Mag-L) respectively. A large body of evidence suggests that AEA elicits a strong anxiolytic response during social interaction. Due to the previous lack of drugs specifically targeting Mag-L, much less is known about the effects of 2-AG on social behavior. Here, we inhibited the degradation of 2-AG using the novel NHS carbamate MJN110, which has a high specificity for Mag-L over FAAH compared to the previous generation of Mag-L inhibitors. We found that the administration of a high dose of MJN110 (5mg/kg) in adolescent male Sprague-Dawley rats produced a significant reduction in total social interaction, pinning, and rearing, and a marginal reduction of nape attack, in the social interaction test. Additionally, we observed a trend toward an increased time spent huddling and increased time spent under the stimulus animal during social interaction. These observations suggest that increased 2-AG signaling may elicit an anxiolytic and serenic response to a social encounter. **Keywords:** Immunohistochemistry, Prefrontal Cortex, Amgydala

16) Dopamine Synthesis in the Ventral Tegmental Area in Rams with High or Low Libido

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Dopamine synthesis and release in the ventral tegmental area (VTA) of the brainstem is credited for pleasurable aspects of certain behaviors such as recreational drug use and mating activity and is central to the reinforcement of those behaviors. It was hypothesized rams lacking overt sexual interest in ewes in estrus would have fewer dopamine synthesizing neurons in the VTA. Rams characterized as having high (n = 4) or low (n = 3) expression of sexual behavior were exposed to urine from ewes in estrus. A second group or high performing rams (n = 3) were exposed to urine from ovariectomized ewes. Following exposure, rams were exsanguinated and brains were preserved by carotid perfusion of 4% buffered paraformaldehyde. The VTA was dissected using surface landmarks, paraffin embedded and sliced at 6 µm. Paraffin embedded slices were stained for tyrosine hydroxylase using immunocytochemistry. Dopamine synthesis of the VTA was influenced ($P = 0.02$) by treatment. Dopamine synthesis was decreased ($P < 0.05$) in the VTA of sexually inactive rams following exposure to a putative sexual stimulus compared to sexually active rams. Dopamine synthesis was diminished ($P < 0.05$) in high sexually performing rams following exposure to urine from ovariectomized ewes, and did not differ ($P > 0.05$) from sexually inactive rams exposed to urine from ewes in estrus. Lack of sexual interest in low performing rams maybe partially a result of decreased dopamine synthesis in the VTA leading to a less pleasurable experience and reduced reinforcement of sexual behavior.

17) Developmental changes in response monitoring ability from age 7 to 25 years with Woody filter technique: An event-related potential study

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Background: Response monitoring, an ability to monitor one's ongoing behaviors and detect errors during task performance, is indicated by an event-related potentials (ERP) component called error-related negativity (ERN). The ERN component is frontally distributed and peaks at approximately 70 milliseconds every time after participants commit an error. Our previous research has demonstrated that children have a smaller ERN amplitude compared to adults. In addition, a quadratic developmental trend in ERN amplitude was found in participants aged 7 to 25 years and reflected the maturation timetable of the anterior cingulate cortex (ACC). Two possible explanations exist for children to display a reduced ERN amplitude compared to adults. One explanation is that response monitoring abilities develop with age. Another explanation may relate to children displaying greater latency jitter in the ERN, the trial-to-trial inconsistency in time intervals of brain responses in incorrect trials. That is, latency jitter might be a potential confounding factor impacting the ERN amplitude. Thus, this study aims to provide a more accurate interpretation of the developmental changes in the ERN amplitude by adjusting latency jitter for all participants. Purposes: The purposes of this study are two folded: (1) Examine the impact of latency jitter on ERN amplitude on participants aged 7 to 25 years (2) Examine the developmental changes in the ERN amplitude in participants aged 7 to 25 years after adjusting for latency jitter. Methods: The EEG data of 238 participants aged 7 to 25 years were collected while participants were performing a two choice flanker task. Both traditional EEG data analysis and Woody filter procedure, a technique that used to reduce the trial-to-trial latency jitter in ERN components, were performed in data analysis. Results: The results demonstrate that a significant interaction between Woody filter technique and chronological age exists. For ERN amplitude without adjusting latency jitter ($M = 12.71$, $SD = 7.01$ µV), chronological age significantly accounted for the variance of ERN amplitude ($F(1,236) = 41.70$, $p < 0.001$, $\eta 2p = 0.15$) and almost approached statistical significance for ERN amplitude after adjusting latency jitter ($M = 23.01$, $SD = 9.22$ µV) ($F(1,236) = 3.58$, $p = 0.06$, $\eta 2p = 0.02$). In addition, curve estimation by regression showed that there was a significant cubic relationship between chronological age on ERN amplitude both for ERN amplitude without adjusting latency jitter ($R2 = 0.16$, $F(3,234) = 17.410$, $p < 0.001$) and for ERN amplitude after adjusting latency jitter ($R2 = 0.04$, $F(3,234) = 2.84$, $p = 0.04$). Discussion: Before adjusting for latency jitter, younger children, especially from age 7 to 11, displayed very small ERN amplitudes. However, after adjusting for latency jitter, ERN amplitudes in young children were significantly larger. Children also displayed significantly more latency jitter than adults, suggesting that children have more inconsistent brain responses when completing a response monitoring task than adults. Possible explanations would

be: (1) Children strategize less efficiently than older participants, or (2) The temporal synchronization in the timing between emergence of ERN component and the button press is poor in children compared to adults. A significant cubic relationship between chronological age and ERN amplitude was found both before and after adjusting for latency jitter, which indicates that the developmental changes exists in ERN amplitude even after adjusting for latency jitter. Implications: This study demonstrated that by adjusting trial-to-trial variability through Woody filter technique in EEG/ERP data analysis, we are able to remove measurement error and provide a more accurate interpretation of the ERN amplitude changes across development. Using this technique allows us to conclude that children display poorer response monitoring abilities and more inconsistent brain responses compared to adults as measured by the ERN. We believe that Woody filter technique can be implemented for other measures of brain response and provide more accurate interpretations of developmental changes and differences seen in clinical populations such as children with attention-deficit hyperactivity disorder (ADHD). **Keywords:** Error-related negativity (ERN), latency jitter, response monitoring, development

18) The Effect of Nicotine Administration and Withdrawal on Sleep in Mice

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Sleep disturbance is a commonly reported symptom during tobacco cessation attempts and is predictive of relapse. Moreover, nicotine alone is reported to disrupt sleep and this effect of nicotine could be a factor in smoking persistence. Despite this knowledge, there surprisingly have been no reported attempts to study the effect of nicotine withdrawal on sleep in an animal model. The current study investigates the effect of nicotine administration and withdrawal on sleep quantity and quality in a forced oral nicotine mouse model. Nine male C57BL/6J mice were implanted with EEG and EMG recording devices using standard procedures. After a recovery and acclimation period, data was recorded continuously for a 4-week period and certain days were chosen over each condition for sleep scoring. Mice had ad libitum access to food and a drinking water solution containing 0.2% saccharin. Baseline sleep and wake data was scored for three consecutive 24 periods, and subsequently averaged. Immediately following baseline, the drinking solution for five of the subjects was changed to 0.2% saccharin supplemented with 200 µg/ml nicotine and nicotine treatment continued for a period of 2 weeks (nicotine group). During this period, the control group continued to receive 0.2% saccharin. EEG/EMG was scored for both test groups on days 1, 4, 8, 11, and 13 of the treatment period. Following the treatment period, withdrawal was initiated spontaneously by excluding the nicotine from the drinking solution and EEG/EMG was scored for the first 2 days of withdrawal. Results indicate that nicotine consumption decreases total sleep. The effect of nicotine on sleep was primarily seen during the lights off period and can mostly be explained by a decrease in time spent in NREM. Additionally, nicotine withdrawal appears to have an effect on the number of stage changes, both the number of sleep stage changes (awakenings) and the number of total stage changes (transitions). These current data suggest that both nicotine consumption and nicotine withdrawal impact sleep, although the measures of sleep affected depend upon whether there is active intake of nicotine or nicotine abstinence. **Keywords:** Sleep, Nicotine, Withdrawal

19) Behavioral Characterization of System xc- Mice

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System xc- is a sodium-independent cystine-glutamate exchanger, taking in one cystine and extruding a glutamate molecule. This transporter may be important in maintaining the balance of extracellular glutamate levels and internal levels of cystine. As a consequence, system xc- has been shown to regulate glutamate receptor function (both iGluRs and mGluRs) through control of extracellular glutamate levels and intracellular glutathione synthesis through cystine import. Thus, we hypothesize that impairments in this transporter may lead to changes in behavior. To test whether system xc- regulates behavior, I chose to examine system xc- mutant mice. Specifically I examined behavior in two different knockouts of the xCT gene (sut and xCT) and a cross of the two strains (xCT/sut) and their respective genetic controls (C3H/He/SnJ, C57BL/6J, B6/SnJ). There was no consistent behavioral phenotype across the two strains of system xc- knockout, or the cross, compared to their controls. Also, male and female xCT/sut mice do not have reduced glutamate levels in the striatum or cerebellum. Homozygous sut mice do not have a reduction in glutamate levels in the cerebellum. Using Western blotting, all three strains of knockout are lacking expression of the xCT protein and do not compensate for glutamate levels by a change in expression of the excitatory amino acid transporters (EAATs). Female mice were also tested in these behavioral tasks and these behaviors are not regulated by changes in the estrus cycle. Therefore it seems that if system xc- regulates behavior it is in a very subtle fashion that is difficult to see with the techniques used in these experiments. **Keywords:** system xc-, behavioral tests, microdialysis

20) Acute Moderate-to-Vigorous Physical Activity Results in Specific Executive Function Gains in Preschoolers

Lisa Schlueter McFadyen-Ketchum, SE Watamura. From the Psychology Department, University of Denver.

Although there is a well-established relationship between physical activity and cognitive function in adults, few studies have examined this relationship in very young children approaching the transition to formal schooling. In a sample of preschool children (N = 81) randomly assigned to an active or passive videogame condition, we examined whether acute moderate-to-vigorous (MVPA) physical activity resulted in improved task performance across six executive function domains (information processing, memory, inhibitory control, task switching, ideation fluency, and task persistence) following each activity condition. Increased physical activity (MVPA condition) resulted in domain specific executive

function gains. Specifically, preschoolers persisted longer in a frustrating task and were able to recall more words following the active (vs. the passive) activity condition. Results demonstrate that short bouts of MVPA may be insufficient to produce global executive function gains in very young children, but may be helpful for enhancing performance in specific school-relevant domains. Therefore, these findings suggest the importance of re-establishing and maintaining MVPA opportunities in school settings. **Keywords:** aerobic, physical activity, executive function, early childhood, school readiness

21) Long-Term Cognitive Impairment from Early Age of Onset and Heavy Marijuana Use

Courtnie J. Paschall, Dr. Joseph Orr. From the Department of Neuroscience, University of Boulder.

As marijuana use and THC content of street-available marijuana rises, it is becoming increasingly important to understand the risks of marijuana use (Cerdá et al., 2012). Our study examines behavioral and self-reported drug history data of 441 subjects, as collected by the Human Connectome Project, to reveal episodic memory (pictorial and verbal), working memory, emotion recognition, executive function, and fluid intelligence differences between marijuana users and non-users. Previous studies have strongly demonstrated short-term neurological impairment in cognitive functions from cannabis use and have linked more subtle, long-term effects to age of first use, number of times used, and duration of use (Crean, 2011; Iverson, 2005; Karila, 2013). Given the size of the available data set, our study will underscore and expand the awareness of long-term cognitive impairment inflicted by early age of onset (under 18 years of age) and heavy use (more than 100 separate sessions from initial use) of marijuana consumption. **Keywords:** Age of Onset, Marijuana Use, Cognitive Disfunction, Human Connectome Project

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22) Increased phasic activation of mesolimbic dopamine neurons promotes negative reinforcement

Noah A. Rauscher, Jacqueline Gallegos, Erik B. Oleson. From the Department of Psychology, University of Colorado Denver.

The mesolimbic dopamine system is a subcortical brain pathway that is highly conserved across vertebrate species. Transient dopamine signals transmitted through this pathway are theorized to promote advantageous action sequences by generating a teaching signal that draws animals toward favorable stimuli and, possibly, away from harmful ones. This teaching signal is generated by high frequency bursts of dopamine neural activity within the midbrain that lead to the release of transient surges in dopamine concentration in terminal regions of the mesolimbic pathway, such as the nucleus accumbens. The current conceptualization of how these transient dopamine signals guide motivated actions focuses almost exclusively on the pursuit of reward. To survive, however, animals must be concerned not only with seeking rewards, but also avoiding aversive events. The role that mesolimbic dopamine plays in avoiding aversive events remains unclear. Our group previously demonstrated that stimuli predicting the avoidance of an aversive event evoke transient surges in dopamine concentration within the nucleus accumbens. Here, we are assessing the causal role of transient dopamine release events in negative reinforcement by harnessing DREADD (designer receptor exclusively activated by an otherwise inert designer drug called clozapine-n-oxide; CNO) technology. We transfected ventral tegmental area dopamine neurons of male heterozygous Th-Cre rats with a Cre-inducible AAV5 viral vector carrying a gene for a Gq-coupled DREADD. Then, we trained rats to avoid electrical footshock (0.75mA) in a signaled operant task until approximately 50% avoidance is observed. We then implant a guide guide cannula aimed at the nucleus accumbens for voltammetric assessment of dopamine and a indwelling jugular catheter, which allows us to monitor real-time changes in dopamine release and control the bioavailabilty of CNO in real time. Finally, we assess for changes in dopamine release events and/or conditioned avoidance induced by either CNO or vehicle treatment, according to a counter-balanced design. We found that CNO-induced activation of Gq-coupled DREADD receptors selectively expressed in dopamine neurons within the ventral tegmental area facilitates signaled operant shock avoidance. These data suggest that transient dopamine release events within the mesocorticolimbic dopamine pathway motivate negative reinforcement, similarly to positive reinforcement. **Keywords:** DREADD virus

23) Sexual orientation is associated with temporal changes in neural processing: An ERP study of in-group bias

Robert L. Ross, Maia T. Nguyen, Stephanie Bastidas, Lucy J. Troup. From the Department of Psychology, Colorado State University.

Biases have been empirically examined within and between social groups for decades. These biases are based on race, age, and sexual orientation to name a few. Behavioral research that has addressed sexual orientation identification suggests that individuals perform better than chance at recognizing the sexual orientation of others. Researchers observed this by examining time processing of face stimuli for same or different sexual orientations. Participants' sexual orientation was also studied in relation to these results. A Neuroscan EEG system fitted with a 64-electrode cap was used

to examine event-related potentials (ERPs), including: the P1, N170, P300, and LPP. The stimulus set included images of self-identified homosexual and heterosexual individuals. Undergraduates completed questionnaires regarding their own sexual orientation, and then completed a sexual orientation identification task for a series faces of varying sexual orientations. Analysis of this data compared homosexual and heterosexual participants' responses to face stimuli that were categorized as either homosexual or homosexual. Results indicated a greater vertex positive potential (VPP) when heterosexual participants viewed heterosexual stimuli at electrode CZ (according to the 10-20 system). The N170, a component closely related to the VPP was reduced at electrode P8 for homosexual participants viewing heterosexual faces. The late positive potential (LPP) was much smaller for heterosexual females who viewed homosexual faces. In-group differences were found in the P300—out-group faces elicited greater P300s at electrodes P3 and P4. These results suggest that face processing is different across sexual orientations, and is in turn dependent on the participants own sexual orientation. These findings are in line with in-group studies and also support an expertise theory of face recognition. **Keywords:** Sexual Orientation, ERP, EEG, Neuroscan, Biases, Behavioral, Face Recognition

24) In vivo optogenetic manipulation of dopamine neurons during cue motivated behavior

Scott Schelp, Jeremy Gage, Greg Krzystynaik, Erik B. Oleson. From the Department of Psychology, University of Colorado, Denver.

The mesolimbic dopamine system is commonly thought to underlie the generation of reward-seeking actions. The mesolimbic pathway originates from A10 dopamine neurons in the ventral tegmental area of the midbrain and projects to brain motivational circuitry—most prominently the nucleus accumbens. Currently accepted theories suggest that transient mesolimbic dopamine release events are involved in assessing the value of reward predictive stimuli and/or in generating motivated action sequences directed toward obtaining reward. During the pursuit of reward, critical associations are formed between the reward stimulus and otherwise neutral stimuli that begin to predict reward availability. Through these experiences, dopamine neurons, which initially represent the receipt of reward, begin to represent the earliest conditioned predictor of reward availability. The resulting concentration of dopamine release scales proportionally to the magnitude of reward predicted. Here, we are investigating the role of cue and reward-evoked dopamine release on cue-motivated behavior. To address this research question we first developed a novel behavioral economics based food-seeking task. In this task, food is provided to rats across 10 different unit-prices (response requirement/reward magnitude). Using fast-scan cyclic voltammetry we determined that cue-evoked dopamine release decreases as a function of price in this task. We next sought to assess the causal role of cue and reward evoked dopamine release in this task using optogenetics. We selectively activated channelrhodopsin-2 expressing dopamine neurons within the ventral tegmentum during either cue or reward presentation. Preliminary data (n=5) reveal that optically facilitating cue evoked dopamine release decreases the maximal price animals will expend for food; whereas, facilitating reward evoked dopamine release increases the maximal price animals will expend for food. It is possible that facilitating cue-evoked dopamine release decreases food motivation in our task because we are violating the animal's expectation (i.e., the animal receives less than expected) and vice versa. Together, these findings suggest antagonistic roles for cue- and reward-evoked dopamine release in influencing cue-motivated behavior. **Keywords:** Optogenetics, Dopamine, Motivation

25) Effects of Stressor Controllability on Prefrontal-Striatal Circuit Activity

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The degree of behavioral control that an organism has over a stressor potently modulates the neurochemical and behavioral consequences of that stressor. Many stress-induced outcomes that occur following uncontrollable stress (e.g., exaggerated anxiety, reduced social exploration) do not occur if the identical stressor is controllable. Furthermore, having control over a stressor (time A) also alters an organism's response to future adverse events (time B), even those that are uncontrollable. Pharmacological studies have identified the prelimbic region (PL) of the medial prefrontal cortex (mPFC) and the dorsomedial striatum (DMS) as necessary for the short and long-term protective effects of behavioral control. Here, we combine PL-to-DMS pathway labeling with fluorescent retrobeads and immediate-early gene immunostaining in order to determine if the dimension of control selectively activates this pathway at time A and/or time B. **Keywords:** stress resilience, anxiety, prefrontal cortex, dorsal striatum, retrograde labeling

26) Single-trial Classification of Error Related Negativity ERN

Fereydoon Vafaei, Chuck Anderson. From the Department of Computer Science, Colorado State University.

Objective: Classification of Error Related Negativity (ERN/Ne) on a single trial basis is a hard problem due to the variability in amplitude and time latency from trial to trial and from subject to subject in BCI experiments. In addition to the subject-specific nature of all types of Event Related Potentials (ERPs), low Signal-to-Noise-Ratio (SNR) of EEG signals, in turn, decreases the accuracy of the classification. In this study, we review, test and compare various approaches and strategies on how to improve the classification process of ERN. Our review consists of comparing several heuristics for pre-processing of EEG raw data as well as comparison of different classifiers used. Approach: Thirty healthy adult subjects' EEG data were recorded while doing Eriksen Flanker tasks. Each subject had errors in approximately 2-20% of the whole trials. Theoretically, erroneous trials should trigger ERN in the brain. We performed an offline pre-processing on the recorded data, including band pass filtering with varying parameters and bandwidth, segmentation and baseline

removal. Then, we trained and tested the performance of the Linear Discriminant Analysis (LDA) with shrinkage, Linear Support Vector Machines (SVM), Ridge Regression and Neural Network (logistic regression) classifiers on the processed data along with fine-tuning the hyper-parameters of the aforementioned classifiers. We also tested non-linear SVM and Quadratic Discriminant Analysis (QDA). Main results: We were able to acquire up to 99.8% accuracy (balanced success rate) in the recognition of ERN of individual subjects on a single trial basis using LDA with shrinkage and linear SVM classifiers. Linear classifiers appeared to get comparable results although LDA outperformed others on average. In contrast, we got lower than or close to random results with non linear SVM and QDA. This may indicate the inherent linearity of the ERN classification problem. We also showed that an efficient selection of the pre processing methods and parameters can significantly improve the accuracy of ERN classification. Significance: Automatic, fast, and accurate recognition of ERN can improve the performance and robustness of the BCI systems in several ways, including removing the barrier of redoing the tasks when the users make errors or when the systems interpret the users' intent wrongly.
Keywords: Error Related Negativity ERN, Classification, Linear Discriminant Analysis LDA, BCI

Developmental

27) Ion channels in Development

Giri Dahal, Sarala Pradhan, Colleen Bartman, Amir Ahmed, Emily Bates. From the University of Colordao, Denver.
Loss of ion channel function during embryogenesis can lead to craniofacial and limb abnormalities, but the underlying mechanism was unknown. We found that ion channel function regulates a novel mechanism of morphogenesis. We found that the Kir2.1 potassium channel regulates BMP release for the proper development of the Drosophila wing and the mouse craniofacial and limb skeleton. **Keywords:** ion channels, membrane potential, Developmental processes, BMP signaling

28) c-Myb and its role in neural development

Coral J. Cabrera Montalvo1, J Vu2, S Vo1, JA Stitzel1. From the 1Institute of Behavioral Genetics, University of Colorado Boulder; and 2Brown University.
c-Myb is a transcription factor associated with areas of neurogenesis in the brain, and has been shown to promote neural progenitor cell proliferation. Whether c-Myb plays a role in neuronal differentiation of the progenitor cells is less clear. To better understand the role of c-Myb in brain development, we have characterized its expression pattern with in situ hybridization in mice of different ages. We show that the highest levels of expression are seen from 15 days of gestation to 7 days old. Throughout development, c-Myb is mostly expressed in the olfactory epithelium, olfactory bulbs, the ventricles, the external capsule, and the pons. Previous studies have indicated that c-Myb is expressed in the hippocampus in the adult brain. However, in situ hybridization failed to detect c-Myb expression in this brain region. To evaluate the impact of c-Myb expression on cell proliferation and neuronal differentiation, we currently are examining the human neuroblastoma cell line SH-SY5Y cell line following transfection with either c-Myb or a control plasmid. Cells also are co-transfected with a GFP plasmid to identify cells that have successfully been transfected. The transfected SH-SY5Y cells currently are being examined for in the effect of c-Myb expression on both proliferation and the extent of differentiation when exposed to retinoic acid. The combination of these studies will provide insight into the role of c-Myb in brain development and neural progenitor cell function. **Keywords:** c-Myb, in situ hibridization, neurodevelopment, fluorescence, transfection, SH-SY5Y, differentiation

29) Amino Acid Transmitter Phenotype of Proopiomelanocortin Neurons in the Arcuate Nucleus of the Hypothalamus During Postnatal Development

Christina S. Dennison and ST Hentges. From the Department of Biomedical Sciences, Colorado State University.
Proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus project to distinct target sites and subsequently release peptides or amino acid (AA) transmitters that induce changes in food intake, energy expenditure, reward pathways, and reproduction. Others have shown that POMC neuron projections exhibit plasticity during the first 3 weeks of postnatal life while the circuitry is being developed. Although previous work in the lab has investigated the distribution of GABAergic and glutamatergic POMC neurons in adult mice, it is not known if the AA phenotype of these neurons exhibits plasticity during early postnatal development. The purpose of this study is to determine if the distribution of glutamatergic and GABAergic POMC neurons change during postnatal development relative to adulthood. To address this question dual fluorescent in situ hybridization (FISH) was used to detect changes in mRNA for the vesicular transporter vGluT2, indicating a glutamatergic phenotype, or changes in mRNA for the GABA synthetic enzyme GAD67, indicating a GABAergic phenotype, in POMC neurons of mice at different ages. Results show increased *vGluT2* expression in POMC neurons on postnatal day 1 (p1) relative to adult mice. This expression in POMC cells steadily declines across development until mice are 8 weeks of age. Similarly, *gad67* expression in POMC neurons appears to be lower at p1 when compared to the expression seen in adult mice. The number of POMC neurons expressing *gad67* mRNA progressively increases from p1 until mice are 8 weeks old. To determine if the proportion of POMC neurons expressing a dual phenotype is higher during postnatal development relative to adult mice dual FISH was used to detect *vGlut2* and *gad67* mRNA in POMCeGFP neurons. Preliminary data suggests a slight increase in POMC neurons expressing a dual AA phenotype during development when compared to adult mice; however, the majority of POMC neurons do not appear to have a dual AA phenotype. Given the importance of AA transmitters released from

hypothalamic NPY/AgRP neurons on food intake, it is likely that AA transmitter release from POMC neurons is also important for maintaining energy balance.

30) Estrogen dependent cell movements in different regions of the developing hypothalamus

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Several nuclear groups in the hypothalamus are characterized by cells that are sensitive to the actions of estrogens. In particular, we have shown previously (Knoll et al., 2007; Eur J Neurosci 26:1091) that cells in the preoptic area-anterior hypothalamus (POA-AH) respond to estradiol by changing their movement characteristics during embryonic development. The current study is extending these observations to include cells in the ventromedial (VMN) and paraventricular (PVN) nuclei. Ongoing experiments use live-cell fluorescence time-lapse microscopy in organotypic brain slices from mice at embryonic day 13(e13) or 14. Cells are observable with yellow fluorescent protein (YFP) driven selectively in neurons under the control of the Thy1 promoter. We are specifically examining the movement responses to three estrogenic agents: estradiol (estrogen receptor (ER) α and β-receptor agonist), propyl pyrazole triol (ERα-receptor agonist PPT), and diarylpropionitrile (ERβ-receptor agonist DPN) in the POA-AH, PVN, and VMN. For labeled cells in each region, 90min of baseline movement is monitored prior to the addition of one of the 3 estrogenic agonists. Movements are then monitored for an additional 90min. Neuronal movement ratio, mean speed, direction change, and mean movement speed are recorded for individual neurons in each of the 3 regions separately. These measurements provide quantitative data for analyzing specific neurons, and demonstrate the ability to track and analyze live migratory networks in these nuclei of the developing mouse hypothalamus. Preliminary data confirms a decrease in movement in response to estradiol in the POA-AH and further suggests a similar action in the PVN and VMN. Interestingly, the α and β-selective analogs indicate effects consistent with ER α (PPT) in the PVN, but not necessarily in POA-AH or VMN. There has been no indication of effects of ER β on cell motions. Additional studies are still necessary to fully characterize these responses, as well as to determine more detailed relationships that might be due to the distance from the third ventricle, and other characteristics of neuronal motion. **Keywords:** neuronal migration, video microscopy, organotypic cultures

31) VIP neurons in the suprachiasmatic nucleus of neonatal mice with disrupted fibroblast growth factor signaling.

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Fibroblast growth factor (Fgf) 8 and its cognate receptor, Fgfr1, are essential for the development of multiple brain regions. Previous studies from our laboratory showed that reduced Fgf8 signaling led to the malformation of neuroendocrine nuclei that originated within the diencephalon, including the oxytocin system in both the paraventricular (PVN) and supraoptic (SON) nuclei. To further understand the role of Fgf8 in the development of other hypothalamic nuclei, we examined if Fgf8 and Fgfr1 deficiencies also impact the integrity of the suprachiasmatic nuclei (SCN). The SCN are principal regulators of the organism's circadian rhythm and consist of neurons that produce vasoactive intestinal peptide (VIP) as main input neurons. The objective of this study is to examine the number of VIP neurons in the SCN of postnatal day (PN) 0 mice hypomorphic for Fgf8, Fgfr1, or both Fgf8 and Fgfr1. Brains were fixed in 4% paraformaldehyde, sectioned in a cryostat, and processed for VIP immunohistochemistry. The number of VIP-immunoreactive (ir) neurons was then quantified in the SCN. Double homozygous (DHom) mice that were homozygous for both Fgf8 and Fgfr1 deficiencies showed a conspicuous absence of SCN as well as SCN VIP-ir neurons. Fgfr1 heterozygous mice, however, showed increased numbers of VIP-ir neurons when compared to wild type (WT) mice, whereas Fgf8 heterozygous mice showed decreased numbers of VIP-ir neurons compared to WT. These data suggest that deficiencies in Fgf8 and Fgfr1 can impact the structural integrity of the SCN via multiple mechanisms.

32) Cellular prion protein promotes olfactory sensory neuron development

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The cellular prion protein (PrPC) has been associated with diverse biological processes including cell signaling, neurogenesis, and neuroprotection, but its physiological function(s) remain ambiguous. The goal of this study is to determine the role of PrPC in adult neurogenesis using the murine olfactory system model. Olfactory sensory neurons (OSNs) within the olfactory sensory epithelium (OSE) undergo neurogenesis, integration, and turnover even into adulthood, making it an ideal model to study neuronal development. Here we determine 1) the role of PrPC in neurodevelopment during homeostasis and 2) the effect of prion-induced neurodegeneration on adult neurodevelopment. To investigate the role of PrPC in OSN proliferation under normal conditions, dividing cells were quantified using the incorporation of the thymidine analog BrdU, using adult wildtype, PrP-overexpressing, and PrP-null mice. Results indicate that PrPC plays a role in maintaining mature OSNs within the epithelium: overexpression of PrPC resulted in greater survival of BrdU-labeled cells within the OSE, whereas absence of prion protein resulted in fewer cells being maintained over time. These results are supported by quantitative PCR (qPCR) analysis of gene expression characteristic of OSN differentiation. In mice overexpressing PrPC, there was an increase in expression of mature neuronal markers, possibly as a result of decreased neuronal turnover. Absence of PrPC had only a modest effect on differentiation, which suggests a redundant role for PrPC in OSN specification. A final neurogenic process required for maintaining OSNs within the OSE

is axons migration out of the OSE and targeting of appropriate synaptic connections in the olfactory bulb (OB). Mice lacking PrPC demonstrated deficits in OSN axon targeting to glomeruli in the OB. These findings support a neuroprotective role for PrPC in adult OSE neurogenesis, whereby more mature neurons are stably maintained in animals expressing PrPC. Using similar methodologies as above, proliferation, maturation, and axon targeting of OSNs were also assessed in prion-infected mice. Current data indicates that a loss of mature OSNs, either as a result of prion neurotoxicity or from deficiencies in OSN maturation, stimulates proliferation of neural progenitor cells and upregulation of OSN development. **Keywords:** Prion infection, IHC, IMF, Western Blot, qPCR, neurodevelopment

Disorders of the Nervous System

33) Elevated neonatal iron intake potentiates progression in the R6/2 mouse model of Huntington's disease
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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder resulting from expression of polyglutamine-expanded huntingtin protein. Brain iron accumulation is implicated in potentiating the neurodegeneration in HD, Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer's disease. Iron levels are also elevated in the brains of HD mice. The purpose of this study was to test the hypothesis that elevated dietary iron intake potentiates HD in the R6/2 mouse model. We tested the effect of nutritionally relevant levels of elevated iron intake during the neonatal period (post-natal days 10-17) and in adult life (5-12 weeks of age). In study 1, neonatal wild-type (WT) and R6/2 HD mouse pups were dosed with vehicle or 120µg/g body weight carbonyl iron/day. HD mice demonstrated poorer performance in spontaneous in-cage wheel running with disease progression. Neuronal cell body size in the striata and cortices of R6/2 mice at 12 weeks were significantly decreased compared to WT controls; there were further decreases in both regions of HD mice with iron supplementation. Levels of oxidized glutathione and lactate, markers of oxidative stress and energetic dysfunction, were increased in the cortices and striata of iron-treated HD mice compared to HD-control mice. In study 2, HD and WT adult mice were fed diets containing 50, 150 or 500 ppm iron from 5-12 weeks. In contrast to the neonatal study we have not found any effect of these different dietary iron levels on HD outcomes. Taken together, our findings show that the neonatal mouse HD brain is vulnerable to elevated iron intake. Early-life iron nutrition may influence onset and progression of human HD. **Keywords:** Huntington's; iron; neurodegeneration; oxidative stress; stereology; neonatal

34) Small Molecule Mimetics of Poly(sialic acid) for Nervous System Regeneration
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Damage to the nervous system due to injury or disease is a significant societal concern. Strategies able to promote regeneration of the damaged nervous system and translate into clinical use remain elusive. Our strategy involves using glycans and mimetics of glycans to promote repair following injury. Glycans are polymerized sugar residues commonly attached to extracellular proteins and lipids. Glycan structure varies tremendously in terms of the monomer units, linkages between monomers and branching structure. Cells of the nervous system are the most extensively glycosylated of all tissues and changes in the state of glycosylation is emerging as a major functional factor underlying development, injury response and the regenerative process. However, glycan expression lacks a predictable template, such as that for DNA-RNA-Protein, and are much more labile molecules compared to proteins. Both of these issues complicate the study of glycans and hamper the ability to use glycans as therapeutic molecules. One of the major efforts of our group is to leverage the neurostimulatory and pro-regenerative features of glycans to promote nervous system regeneration. One of the ways we are pursuing this is to identify and validate small molecules that may mimic the structure and activity of glycans. In a screen for mimetics of poly(sialid acid) (PSA), several compounds were identified, including tegaserod. Tegaserod is a FDA-Approved Drug for treating irritable bowel syndrome by stimulating 5-HT4 receptors on enteric neurons. We found that tegaserod has a second mechanism of action as a mimetic of PSA and stimulates regeneration of the nervous system in animal models. Tegaserod is just one of many candidate mimetics and we have significantly expanded this effort for other glycans we hypothesize may promote repair of the damaged nervous system.

35) Comparison of phospho-tau and cofilin-actin rod pathologies in the brains of subjects from a longitudinal aging study
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The presence of extracellular amyloid plaques composed mainly of fibrils of the β-amyloid peptide (Aβ), as well as intracellular neurofibrillary tangles composed mainly of hyperphosphorylated tau protein, is used for post-mortem confirmation of the diagnosis of Alzheimer's disease (AD). However, it is generally accepted that soluble forms of Aβ oligomers and not fibrils, which are deposited in plaques, are most responsible for the synaptic loss and eventual neuronal death that accompanies AD progression. In cultured mammalian neurons, treatment with soluble forms of Aβ most relevant to AD induces in many neurons the formation of cofilin-actin rods. Rods may grow to occlude the neurite and

block transport, leading to loss of microtubules and synapses. Tau is a microtubule binding protein whose hyperphosphorylation depends upon its release from microtubules. Thus, rod formation might play a role in the loss of synapses and the development of tau pathology in AD. Although neurofibrillary tangles are very large and thus easily observed, most tau pathology is in the form of neuropil threads, deposits of the hyperphosphorylated tau within neurites. To determine if cofilin-actin rods might play a role in AD progression, we obtained pieces of frontal cortex and the hippocampal formation from nearly identical regions of multiple subjects who were part of a longitudinal study and thus could be grouped as non-cognitively impaired (NCI), mild cognitively impaired (MCI), early AD (eAD), or mid to late AD. All samples were obtained with a short postmortem interval before fixing in 4% formaldehyde (ave 2.5 h but all less than 4 h). The average age of subjects in each group was between 86 and 89 years. We prepared 30 μm sections of cortical and hippocampal tissue, and after immunofluorescence staining for cofilin and phosphorylated tau protein, quantified rod numbers, rod areas and neuropil thread areas in brain sections from each subject. Rods in the hippocampal formation were most prevalent in the entorhinal cortex, the first brain region to show pathology during development of AD. No significant rod numbers were observed in non-cognitively impaired subjects but a few MCI subjects showed rods increased more than tau pathology, which was more prevalent in mid- to late AD subjects. Comparison of rod and neuropil thread pathology in the Frontal Cortex is ongoing. Our current results appear to indicate rods may play a contributing factor in AD progression, particularly in early stages. (Supported in part by NIH grant AG044812 (JRB), a CSU Core Infrastructure Grant, and generous donations to our Development Fund)

36) Exploring the role of prion protein expression level and profile on pathogenesis of chronic wasting disease

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Background: Transgenic (Tg) mouse models expressing cervid prion protein (PrP) have been crucial to our understanding of chronic wasting disease (CWD). However, the utility of current models for studying peripheral pathogenesis of disease has been limited due to variable transgene expression of PrP outside the central nervous system. Furthermore, analysis of disease progression is complicated by five-fold overexpression of PrP in brains of our Tg models. To improve upon our model system, we have generated constitutive knock-in mouse models expressing either deer or elk PrP under the control of the endogenous murine promoter. Results: Targeting vectors were designed to replace the mouse Prnp open reading frame (ORF) with the ORF from either deer or elk PRNP. Knock-in ES cells expressing the constitutive knock-in allele were microinjected into C57BL/6 and highly chimeric mice were selected for breeding with FVB females. Passage of the knock-in allele to germline was screened by coat color and confirmed by genotyping. Pnrp expression level in the brain was measured by RealTime-PCR and protein levels in the brain, heart, lungs, liver, spleen and kidney were compared between age and sex matched wild-type FVB mice and knock-in elk and deer mice by Western blotting. Inoculation of CWD prions into knock-in mice confirmed CWD prions originating from elk have a more rapid incubation time and unique neuropathology in comparison to isolates from deer. In addition, our new model is susceptible to peripheral routes of inoculation including intraperitoneal and oral. Conclusions: Constitutive knock-in animals express cervid PrP under the control of the endogenous mouse promoter and express physiologically relevant levels of cervid PrP in the appropriate tissues. This novel model reveals a species barrier between deer and elk based solely on a single amino acid change between deer and elk prion protein. We were also able to model natural transmission of CWD and assess tissue tropism related to route of exposure.

37) Brain indoleamine-2,3-dioxygenase enzymatic activity is increased in the N171-82Q mouse model of Huntington's disease

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by motor impairment and cognitive decline. In addition to neuronal atrophy and loss, HD brains demonstrate pre-clinical neuroinflammation. Enhanced tryptophan degradation through the kynurenine pathway is part of the neuroinflammatory response. Neurotoxic intermediates generated in this pathway have been implicated in HD progression. The oxidation of L-tryptophan to L-kynurenine (kyn), catalyzed by the enzyme indoleamine-2,3-dioxygenase (IDO), is the first and rate-limiting step in the kynurenine pathway. IDO activity may therefore be an important marker of neuroinflammation and progression of HD. Kynurenine/tryptophan ratios, pathway metabolite concentrations and enzyme transcript levels have been used as an indicator of kynurenine pathway activity. However, we sought to more specifically quantify the increased enzymatic activity suggested by these outcomes. The goal of this study was to develop an IDO enzymatic activity assay and measure IDO activity in HD mouse brain. We use an end-point assay based on measurement of kyn after incubation of soluble brain protein extract with an excess of L-tryptophan. Using this method, we quantified IDO activity in cerebral cortex and striatum of 14-week old female N171-82Q HD mice and wild-type litter mates. Enzymatic activity, measured as micromoles kyn/micrograms brain protein/minute, was significantly increased in HD mice compared to wild-type litter mates. Here we show activation of the kynurenine pathway in the brains of N171-82Q HD mice at an age corresponding to early-advanced disease. The enzymatic activity of IDO will be a valuable marker to use in studies aimed at addressing the role of neuroinflammation and inflammation-modulating interventions HD mice. Funding: NIH P30GM103398 (Neuroscience Core Center). **Keywords:** Neurodegeneration, Neuroinflammation, Immunology, Biochemistry

38) 1,1-bis(3'indolyl)-1-(p-chlorophenyl)methane (C-DIM12) provides dopaminergic phenotypic stability through Nurr1 interactions in neuronal cultures

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Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons of the ventral midbrain. Protein aggregation, oxidative stress, and neuroinflammation are important pathogenic mechanisms that promote neuronal loss. In previous studies, the orphan nuclear receptor Nurr1 (NR4A2) has been shown to suppress inflammatory gene expression in glial cells and also regulate many genes associated with the production/release of dopamine (DA) in neurons. Nurr1 is also critical for DA neuron development and homeostasis and is down regulated in PD patients. Despite the many known functions of Nurr1, an endogenous ligand has yet to be discovered. The phytochemical-based compound, 1,1-bis(3′-indolyl)-1-(p-chlorophenyl) methane (C-DIM12) has been shown to activate Nurr1 in cancer cells and demonstrated neuroprotective efficacy by preserving tyrosine hydroxylase (TH) positive neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD in mice. In the present study, we examined the capacity of C-DIM12 to induce expression of Nurr1-regulated genes in two dopaminergic neuronal cell lines (N2A, N27) in order to determine whether pharmacologic activation of Nurr1 could support a dopaminergic phenotype and provide neuroprotection against neurotoxic injury by 6-hydroxydopamine (6-OHDA). mRNA quantification by qPCR showed modest induction of Nurr1 expression in two neuronal cell lines and significantly induced Nurr1-regulated genes with C-DIM12 in a time- and dose-dependent manner. Knockdown of Nurr1 expression by RNAi prevented C-DIM12-mediated induction of regulated genes, including TH and VMAT2, indicating that Nurr1 is required for gene activation by C-DIM12. C-DIM12 also increased expression of Nurr1 in N2A cells overexpressing human Flag-tagged Nurr1, as well as increased cell viability following exposure to 6-OHDA. Collectively, these data suggest that selected C-DIM structures are functional activators of Nurr1 in dopaminergic neurons and could therefore represent a novel neuroprotective strategy.

39) Thioltransferases TXN1 and TXNDC10 protect against neuronal atrophy in a lentiviral mouse model of Huntington's disease

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Huntington's disease (HD) is a progressive neurologic disorder caused by polyglutamine-expanded mutant huntingtin protein (mhtt). Mutant hungtingtin protein forms oxidative and thiol-dependent oligomers whose rate of degradation is slower than monomeric mhtt. We have hypothesized that a thiol transferase exists that converts oxidized mhtt oligomers to monomeric protein and may thereby decrease mhtt levels. We screened a number of thiol transferase enzymes by co-transfection with plasmids encoding N171-40Q huntingtin and thioltransferases into COS1 cells and measured mhtt levels 48 hours later by Western blot analysis. The primary screen revealed that the thioltransferases thioredoxin 1 (TXN1) and thioredoxin domain-containing protein 10 (TXNDC10) decreased total soluble mhtt. In a secondary screen we expressed enzymatically active / inactive versions of TXN1 and TXNDC10 with N171-40Q huntingtin. We confirmed that TXN1 and TXNDC10 decreased N171-40Q huntingtin levels in COS1 cells. We subcloned these two genes into a lentiviral vector that uses the phosphoglycerate kinase promoter. We tested their potential protective effects in mouse HD by co-injection of lentiviruses expressing the following combinations of genes: WT control (N171-18Q + inactive TXN1 or TXNDC10), HD control (N171-82Q + inactive TXN1 or TXNDC10) and HD treated (N171-82Q + active TXN1 or TXNDC10). Lentivirus was delivered by unilateral intra-striatal injection at 8 weeks of age in B6/C3H F1 female mice; mice were sacrificed at 16 weeks of age. There was no effect of the treatments on behavioral outcomes. However, the HD control group had significantly smaller striatal neuronal cell bodies than the WT control group as determined by confocal stereology; this effect was reversed in the HD treated groups by both TXN1 and TXNDC10. Therefore, TXN1 and TXNDC10 provide therapeutic benefit in this lentiviral model of HD. **Keywords:** subcloning, cell transfection, confocal microscopy, stereology

40) HSPB8: A candidate susceptibility factor in prion diseases

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Transmissible spongiform encephalopathies (TSEs) or prion diseases are transmissible, fatal, neurodegenerative diseases. These diseases are associated with the accumulation and aggregation of the prion protein in the brain followed by irreversible loss of neurons. However, the cellular factors responsible for the accumulation, misfolding, propagation, and the resultant pathogenesis are not understood. To address this we engineered rabbit kidney RK13 cells, essentially null for PrP expression, to express PrP from different species. While chronically infected clones were established, a proportion of clonal derivatives subsequently lost infection. Although the majority of clones that lost infectivity were susceptible (S) to reinfection, a subset was resistant (R), despite expressing cellular PrP. We hypothesize that this phenotypic distinction reflects genetic differences between S and R subclones. To test this we have used microarray and RNA sequencing (RNAseq) analyses to identify candidate genes involved in this process. Expression differences of various cellular factors have been identified, leading to candidate genes, like HSPB8, that we are in the process of validating and analyzing further using real-time PCR and western blotting. The consistent up regulation of HSPB8 in S compared to R cells makes this chaperone a candidate susceptibility factor. While chaperones like HSPB8 regulate the proper folding of proteins in normal conditions, during cellular stress, proteostatic mechanisms are activated to refold,

inhibit or degrade misfolded proteins. Previous work has shown that HSPB8 is up regulated in the brains of patients with protein folding disorders (Seidel et al., 2012), indicating a role for this chaperone in numerous neurodegenerative diseases. HSPB8 also acts in a pathway that inhibits protein synthesis through the phosphorylation of eIF2α (Carra et al., 2009), a key component of the unfolded protein response (UPR). My previous work showed that activation of the UPR is detrimental during prion disease, but that targeting this pathway prevents prion induced neurodegeneration (Moreno et al., 2013; Moreno et al., 2012). Following validation that HSPB8 is up regulated in S versus R cells, we examined expression levels of HSPB8 in various prion disease settings. We identified an early increase of HSPB8 protein levels in mice infected with the RML strain of prions, as well as in diseased transgenic mice expressing a genetic mutation associated with the inherited human prion disease Gerstmann-Straussler Scheinker (GSS) syndrome. Further analysis of HSPB8 and its downstream pathways will be examined using in vitro and in vivo approaches to understand the mechanism by which HSPB8 functions in prion disease and other protein misfolding disorders of the brain. **Keywords:** prion disease, molecular chaperones, unfolded protein response

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41) The effects of exercise on methamphetamine-induced serotonin, tyrosine hydroxylase, and dopamine neurotoxicity in Rattus norvegicus

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Abuse of methamphetamine (METH) in the Unites Stated has increased significantly in the past 15 years, and use is now endemic the Western states. Colorado currently ranks 7th in the nation for total number of METH users over the age of 25. In addition to the known health risks associated with psychostimulant abuse, METH carries the additional danger of permanent brain injury. One well-known animal model of METH use utilizes binge METH administration, where repeated doses of METH are given to rats in a single day. This dosing regimen has been shown to cause long-lasting damage to dopaminergic nerve terminals and serotonergic nerve terminals similar to that seen in human METH abusers. In humans, it has been suggested that METH-induced monoaminergic damage may lead to the development of Parkinson's Disease. Exercise is a non-pharmacological treatment being explored for use in treating Parkinson's Disease and this work has recently been extended to the study of METH-induced monoaminergic neurotoxicity. It has been shown that when rats exercised for 3 weeks before and 3 weeks after a binge treatment of METH, this exercise significantly attenuated METH-induced decreases in striatal dopamine. Interestingly, if the exercise regimen was limited to only 3 weeks before a binge treatment of METH, it did not protect against striatal DA damage. This suggests that pre-METH exercise does not help with prevention of neurotoxicity, but perhaps post-METH exercise aids in recovery. This study specifically tested the effects of 3 weeks of exercise after a METH binge on the recovery of dopaminergic nerve terminals in the striatum and serotonergic nerve terminals in the prefrontal cortex.

42) Indoleamine-2,3-dioxygenase Activity is Modulated by Iron Status: implications for neurodegenerative diseases

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Neuroinflammation and elevated brain iron are implicated in the pathogenesis of neurodegenerative disorders including Alzheimer's (AD), Huntington's (HD), and Parkinson's diseases (PD) and amyotrophic lateral sclerosis (ALS). The proinflammatory cytokine, interferon-gamma (IFN-g) is increased in these conditions and upregulates the enzyme indoleamine-2,3-dioxygenase (IDO). IDO catalyzes the oxidation of tryptophan to kynurenine, the first and rate-limiting step in the kynurenine pathway of tryptophan degradation. Activation of this pathway results in the generation of neurotoxic metabolites. The long-term goal of this project is to test the hypothesis that elevated brain iron may potentiate the tryptophan-degradation pathway via stimulation of IDO. Here we tested the effect of iron modulation on IDO activity in vitro. We first validated an IDO activity assay based on HPLC-MS/MS detection of kynurenine. We then tested the effect of iron chelators and elevated iron levels on IDO activity in brain homogenates. We show that IDO enzymatic activity is increased in response to in vitro addition of iron and inhibited using an iron (II) chelator. These findings indicate a putative molecular link between elevated brain iron and neuroinflammation. This study was supported by a pilot grant under NIH P30GM103398 (Neuroscience Core Center). **Keywords:** Neurodegeneration, Neuroinflammation, Biochemistry

43) Alphavirus-manganese interactions and dopaminergic neurodegeneration

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Viral infection is implicated as a possible risk-factor for neurodegenerative diseases, including Parkinson's disease (PD). Infection with neurotropic alphaviruses can produce many of the same long-term degenerative effects seen in PD, including protein aggregation, increased levels of oxidative stress, autophagy/mitophagy defects, neuroinflammation, and neuronal death. Neurotropic viruses may therefore represent a better animal model for studying gene x environment interactions in PD that recapitulate more features of the human disease than drug-based lesioning models or genetic models that often lack a pronounced phenotype in the substantia nigra. Evidence suggests that viral infection may act in synergy with other recognized risk-factors, such as aging, genetic factors, and/or previous exposures to environmental neurotoxins to promote neurodegeneration. Among neurotoxins that could have a pronounced gene x environment interaction with neurotropic viruses, Manganese (Mn) is of interest because excessive exposure early in life can have lasting effects on neurological function and can also enhance the neurovirulence of alphaviruses. We are developing a novel method for testing gene x environment interactions in parkinsonism using a neurotropic alphavirus expression system (AES). Following convenient intranasal inoculation, 100% of animals become infected and the progression of infection can be non-invasively monitored in situ using luciferase-expressing viruses and whole body bioluminescence imaging. Neuroinvasion occurs through olfactory sensory neurons and the infection spreads along the neuronal axis in a pattern that mimics the Braak-staging system of PD. The severity and persistence of viral infection can be tightly controlled and our preliminary data using unbiased stereology indicate that AES infection results in significant loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). We aim to determine if pre-exposure to Mn during juvenile development will enhance susceptibility to viral-mediated loss of dopaminergic neurons, in part through an increased neuroinflammatory response. We expect that these studies will demonstrate that the proposed AES-based model is a powerful method for inducing parkinsonism in mice, as well as for transgene delivery into the CNS, that can be used to identify novel gene x environment interactions relevant to PD. Additionally, we expect that exposure to Mn during juvenile development will exacerbate neurodegeneration following adult infection with WEEV. The results of these studies will increase our understanding of the environmental links to neurodegenerative disease and will provide a powerful new animal model for studying virus/toxin interactions in the CNS. **Keywords:** Parkinson's disease, neurodegeneration, viral infections of the CNS, CLARITY tissue-transmutation, manganism

44) Activation of the nuclear receptor Nur77 by a novel diindolylmethane analog suppresses inflammatory gene expression in primary astrocytes

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Inflammatory activation of glial cells is involved in the progressive loss of dopaminergic neurons in Parkinson's disease (PD). Astrogliosis is accompanied by activation of the transcription factor, Nuclear Factor-kappa B (NF-κB), which coordinately regulates the expression of multiple neuroinflammatory genes associated with PD including inducible nitric oxide synthase (iNos), tumor necrosis factor alpha (Tnfα), and interleukin 1β (Il1β). These observations suggest that inhibition of NF-κB in glial cells could be a promising therapeutic target for the prevention of neuroinflammatory injury. Nuclear orphan receptors in the NR4A family, including NR4A1 (Nur77) and NR4A2 (Nurr1), are reported to antagonize the effects of NF-kB on inflammatory gene expression. However, high affinity pharmacologic ligands of these receptors have been lacking. A novel ligand of Nur77, 1,1-bis (3'-indolyl)-1-(p-methoxyphenyl) methane (C-DIM5), activates Nur77 in cancer cells and causes nuclear degradation of the transcriptional co-activator C/BP (p300), which is also required for the transcriptional activity of NF-κB. We therefore postulate that activation of Nur77 by C-DIM5 in astrocytes would suppress NF-κB-dependent inflammatory gene expression induced by the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) and the inflammatory cytokines interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α). C-DIM5 increased expression of Nur77 mRNA and suppressed expression of neuroinflammatory genes. C-DIM5 also inhibited the expression of multiple NF-κB-regulated inflammatory and apoptosis genes in qPCR array studies but did not prevent p65 translocation to the nucleus, suggesting a nuclear-specific mechanism of inhibition. These data demonstrate that C-DIM5 prevents the production of neurotoxic inflammatory mediators in glial cells through inhibition of NF-kB, suggesting that this series could be a useful modality in preventing neuroinflammation.

45) Drosophila p38 MAP Kinase Regulates Age-dependent Protein Homeostasis

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Maintaining a properly balanced proteome is an important aspect of cellular health and function. Disruptions in protein homeostasis, such as aging or exposure to oxidative stress, can lead to the accumulation of damaged or misfolded proteins, which in turn can form protein aggregates. These aggregates are thought to be toxic and potentially lead to an aged or diseased state, such as Alzheimer's disease, Parkinson's disease and ALS. Therefore it is critical for these damaged proteins to be properly degraded or cleared from the cell. One protein quality control mechanism is the Chaperone Assisted Selective Autophagy (CASA) complex which targets damaged proteins for destruction via the

autophagosome/lysosome. The Drosophila CASA complex consists of the chaperones HspB8 and Hsc70 and the nucleotide exchange factor, Starvin. We find that HspB8 physically interacts with the p38 MAP Kinase (p38K), which we have previously shown to regulate aging and oxidative stress. In addition, we find that p38K colocalizes with CASA complex members at the Z-disk of the adult flight muscle and are testing these interactions in the adult brain. We also find that p38K regulates protein homeostasis in response to natural aging and oxidative stress. Finally, we find that p38K genetically interacts with the CASA complex to regulate lifespan and protein homeostasis. As p38K has been implicated in many age-dependent neurodegenerative diseases, our results suggest that altered p38K function may contribute to the accumulation of protein aggregates, potentially leading to a disease state. **Keywords:** Oxidative stress, aging, protein aggregation

46) Emerging Roles of Synaptotagmin: Modeling Neurogenic Disorders in Drosophila
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Synaptotagmin, a synaptic vesicle protein, is widely known as the fast, synchronous Ca++ sensor that mediates neurotransmitter release. It's C2B Ca++ binding domain has been extensively analyzed for its essential role in triggering synaptic vesicle fusion in many animal models. Due to its essential nature, many synaptotagmin mutations result in early lethality in these animal models. Recently, whole-exome sequencing has demonstrated that mutations in synaptotagmin are associated with human disease. In two families, multigenerational dominant deficits have been linked to single adjacent point mutations in synaptotagmin's C2B domain. These dominant mutations are located in a highly conserved sequence required for Ca++ binding, and the patients present with symptoms similar to Lambert-Eaton myasthenic syndrome (LEMS). With a view to identifying the molecular mechanisms underlying the human phenotype, we have generated an homologous point mutation in the C2B domain of Drosophila synaptotagmin.

47) PrPC-dependent cofilin-actin rod formation occurs through neurite-specific activation of NADPH oxidase
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The cellular prion protein, PrP^{C}, mediates β-amyloid (Aβ) and proinflammatory cytokine (e.g. TNFα) signaling to activate NADPH oxidase (NOX) and generate reactive oxygen species (ROS). Once oxidized by ROS, disulfide cross-linked cofilin induces formation of filaments containing 1:1 cofilin:actin into rod-like bundles (rods). Rods sequester the majority of cofilin within the neurites in which they form, blocking transport and delivery of materials for synapse maintenance. PrP^{C}-dependent rod formation occurs in a subset of rodent hippocampal or cortical neurons (~20%), and in a subset of neurites (~45%) from these neurons. It is only within these rod-containing neurites that cofilin dephosphorylation (activation) increases. Here we utilized $p47^{PHOX}$roGFP as a redox probe for measuring NOX-2 activity to localize neuritic ROS in response to either Aβd/t or TNFα. We also show that the HIV viral envelope protein, gp120, induces rods and neuritic ROS in a similar manner to other PrP^{C}-dependent rod stimuli. Overexpression of PrP^{C}-EGFP in the absence of other treatments induces rods in up to 40% of neurons, but the percentage of neurites forming rods does not change significantly. These results suggest PrP^{C} may be limiting to the neuronal population response, but that some other factor limits the response to specific neurites. Removing inducers or inhibiting NOX activity in cells containing PrP^{C}-dependent rods leads to their disappearance with a half-life of ~36 minutes. The transient nature and PrP^{C} dependency of rods induced by diverse stimuli suggests that membrane microdomains containing PrP^{C} may recruit the oxidizing machinery necessary to initiate and sustain rod formation. This hypothesis is supported by the inhibition and reversal of PrP^{C}-dependent rods and the increase in ROS by the naturally occurring plant triterpene, ursolic acid (UA), and the pharmacological peptide RAP310, an oral analog of D-ala1-peptideT-amide (DAPTA). UA and RAP310 interfere with membrane architecture permissible for lipid raft coalescence. The vast majority of neurodegenerative disorders, such as Alzheimer disease, are considered sporadic in incidence and multifactorial in cause, making treatment at an early stage a significant challenge. If cofilin-actin rods indeed bridge multiple disease initiating mechanisms into a common pathway leading to synapse loss, they provide a valuable target for therapeutic intervention. (Supported in part by NIH grant AG044812 to JRB, a CSU Core Infrastructure Grant, and from generous donations to our Development Fund).
Keywords: Alzheimer disease, prion protein signaling pathways, reactive oxygen species, lipid raft domains

Neural Excitability, Synapse, and Glia

48) Mitochondria mediate local stimulation of L-type calcium channels in cerebral artery smooth muscle
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The mitochondrial respiratory chain is the primary source of ATP in eukaryotic cells. Reactive oxygen species (ROS), which can influence protein activity via thiol oxidation, are a byproduct of mitochondrial respiration whose production can be modulated by local calcium and ROS microdomains. These parameters allow mitochondria to participate in various intracellular signaling pathways including transcription modification, apoptosis, and modification of electrophysiological activity. Our laboratory has recently demonstrated the angiotensin II- dependent stimulation of L-type calcium channels in cerebral resistance arteries occurs through local redox- dependent activation of PKCα. The resultant persistent gating of a discreet subpopulation of L-type calcium channels creates ROS- and calcium- enriched subplasmalemmal microdomains in which local mitochondria could potentially participate. We therefore tested the hypothesis that local mitochondrial ROS modulate angiotensin signaling in cerebral artery smooth muscle and may therefore serve as a therapeutic target in vascular dysfunction. First we determined the existence of a plasmalemmal subpopoulation of mitochondria using a combination of confocal and Total Internal Reflection Fluorescence Microscopy (TIRFM). When stimulated with angiotensin II, significant colocalization between these mitochondria and plasmalemmal calcium influx sites was observed in live, isolated myocytes. When mitochondrial ROS production was inhibited either through increased ROS scavenging or acute rotenone application, angiotensin II exposure failed to result in persistent L-type channel gating. Mitochondrially targeted ROS scavenging was likewise able to attenuate endothelial independent angiotensin- mediated arterial constriction and blunt the development of hypertension in- vivo using the LNAME model of endothelial dysfunction. **Keywords:** calcium signaling, redox physiology,live cell imaging, electrophysiology, TIRFM, confocal microscopy,

49) Fasting increases agouti-related peptide neuron inhibition of proopiomelanocortin neurons in the hypothalamus

Matthew S. Dicken, AR Hughes, ST Hentges. From the Department of Biomedical Sciences, Colorado State University.
Although early studies attributed the ability of neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons to stimulate food intake and increase energy balance to the release of their peptide transmitters, recent findings indicate a critical role for the co-release of the inhibitory amino acid transmitter GABA from these neurons. Under conditions of energy deficit, action potential frequency increases in NPY/AgRP neurons, and this correlates with increased GABA tone onto hypothalamic proopiomelanocortin (POMC) neurons, which would be expected to quiet the anorexigenic POMC neurons and further increase food intake. However, it is not clear whether NPY/AgRP neurons are the primary source of GABA released onto POMC neurons, or whether NPY/AgRP neurons are the predominate cells that undergo an increase in GABA tone upon fasting. The present study utilized fluorescent in situ hybridization and optogenetic approaches to address these questions. The results show that an overnight fast causes a selective increase in the mRNA for the GABA synthetic enzyme GAD67 in NPY/AgRP neurons, and this correlates with an increased probability of GABA release from NPY/AgRP neurons onto POMC neurons, as well as an increase in the readily releasable pool of GABA in NPR/AgRP neurons. Also demonstrated is that an increase in GABA release from NPY/AgRP neurons alone is sufficient to inhibit action potentials in POMC neurons. Altogether, it appears that NPY/AgRP neurons can potently regulate the excitability of POMC neurons, and presumably other target neurons, in an energy-state-dependent manner, and that up-regulated Gad67 expression can be a reliable indicator of increased GABAergic transmission. **Keywords:** Optogenetics, electrophysiology

50) A novel role of adenosine A2B receptors in regulating calcium-activated small conductance potassium (SK) channels at the synapse

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After ischemic injury adenosine levels are highly elevated in the brain. The role of adenosine receptors in ischemic injury has been studied, however very little is known about the A2B adenosine in the brain. In peripheral organs ischemia-reperfusion causes the upregulation of A2B receptors and organ protection. In contrast, we made the remarkable observation that inhibition of A2B receptors provides robust protection against stroke injury in mice. Therefore, we used electrophysiology methods to explore potential mechanisms to explain the injurious role of A2B receptors in brain ischemia. Patch-clamp recordings from adult mouse hippocampus demonstrate that pharmacologic activation of the A2B receptor leads to a reduction in SK channel mediated current. Synaptic recordings implicate this A2B receptor regulation of SK channels is present at the synapse. These findings propose a novel signaling interaction between A2B receptor signaling and SK channel functional activity, which may provide an improved understanding of SK channel regulation at the synapse. Further, these observations may have implications in neuronal excitability and efficacy of synaptic transmission, particularly after ischemic injury. **Keywords:** SK channels, Adenosine A2B receptors, Electrophysiology, Brain Ischemia, Stroke

51) Linking Aβ42-Induced Hyperexcitability to Neurodegeneration, Learning and Motor Deficits, and a Shorter Lifespan in an Alzheimer's Model

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Alzheimer's disease (AD) is the most prevalent form of dementia in the elderly. β-amyloid (Aβ) accumulation in the brain is thought to be a primary event leading to eventual cognitive and motor dysfunction in AD. Aβ has been shown to promote neuronal hyperactivity, which is consistent with enhanced seizure activity in mouse models and AD patients. Little,

however, is known about whether, and how, increased excitability contributes to downstream pathologies of AD. Here, we show that overexpression of human Aβ42 in a Drosophila model indeed induces increased neuronal activity. We found that the underlying mechanism involves the selective degradation of the A-type K+ channel, Kv4. An age-dependent loss of Kv4 leads to an increased probability of AP firing. Interestingly, we find that loss of Kv4 alone results in learning and locomotion defects, as well as a shortened lifespan. To test whether the Aβ42-induced increase in neuronal excitability contributes to, or exacerbates, downstream pathologies, we transgenically over-expressed Kv4 to near wild-type levels in Aβ42-expressing animals. We show that restoration of Kv4 attenuated age-dependent learning and locomotor deficits, slowed the onset of neurodegeneration, and partially rescued premature death seen in Aβ42-expressing animals. We conclude that Aβ42-induced hyperactivity plays a critical role in the age-dependent cognitive and motor decline of this Aβ42-Drosophila model, and possibly in AD.

52) Exploring the role of caudal brainstem proopiomelanocortin neurons in feeding circuits

Alexander R. Hughes, MS Dicken, ST Hengtes. From the Department of Biomedical Sciences, Colorado State University.

Proopiomelanocortin (POMC) neurons are a critical component of metabolic regulation and affect aspects of energy expenditure, food intake, and reward. POMC neurons are found in two distinct populations of the brain: the arcuate nucleus (ARC) of the hypothalamus and the nucleus tractus solitarius (NTS) of the caudal brainstem. Hypothalamic POMC neurons are capable of acting slowly to affect metabolic changes through the release of its peptides, or more rapidly by the release of the amino acid neurotransmitters, glutamate and gamma-aminobutyric acid (GABA). Unlike hypothalamic POMC neurons, NTS POMC neurons do not produce detectable levels of peptide, although these neurons are responsive to metabolic indicators such as afferent vagal stimulation, cholecystokinin (CCK), and leptin. Previous studies suggest that NTS POMC neurons are involved in a short term satiety pathway. We hypothesize that the primary actions of these NTS POMC neurons are mediated through the release of amino acid transmitters given the apparent absence of peptide production. To examine the amino acid phenotype of these neurons we used fluorescent in situ hybridization (FISH) to detect the presence of mRNA coding for precursor molecules that allow for GABA and glutamate to be released. Specifically, mRNA for the cleavage enzymes Gad65 and Gad67 was detected as an indicator of GABAergic neurons, while the vesicular transporters vGlut1 and vGlut2 were indicative of glutamatergic neurons. We found that 39% of neurons expressing gfp driven by the POMC promoter also expressed vGlut2, 28% expressed Gad65, and 14% colocalized with both vGlut2 and Gad65 in the same cell. Further, experiments combining optogenetics and electrophysiology experiments demonstrated physiological release of both amino acid neurotransmitters by NTS POMC neurons. Altogether, it appears that amino acid transmitters provide the primary signals contributing to NTS POMC neurons involved in energy balance regulation.

53) Layer Dependent Dopaminergic D1 receptor activation effects on frequency dependent short-term synaptic plasticity

Jonna M. Leyrer-Jackson, Mark P. Thomas. From the School of Biological Sciences, University of Northern Colorado.

Executive functions (e.g. working memory, attention, planning abilities) are known to be mediated by prefrontal cortical areas of the human brain which share homology with mouse medial prefrontal cortex (mPFC). It is well known that working memory (WM) tasks are associated with rhythm generation and WM deficits are known to be accompanied by changes in rhythmic activity. Neuronal oscillations within the theta frequency band have been extensively linked to WM function, and are enhanced during tasks involving WM. Recent studies have revealed that intrinsic properties of neuronal elements and synaptic connectivities (excitatory and inhibitory) contribute to rhythm generation. Here we focus attention on the excitatory synaptic components mediated by AMPA and NMDA receptors. NMDA receptor mediated transmission relies heavily on coincidence detection. A dominant hypothesis is that rhythms play a large role in synchronizing timing events important for NMDAR-mediated synaptic plasticity. Cortical circuits are known to process information from different sources within different layers of the cortex, which leads to different rhythmic dynamics. Dopaminergic neurons innervate the prefrontal cortex and are known to play a role in encoding, updating and maintaining WM processes. Additionally, it is thought that dopamine may influence rhythm generation through actions on intrinsic and voltage-dependent ion currents, and may have differential effects on cortical layers. Due to the importance of dopaminergic modulation of the prefrontal cortex, and the strong presence of rhythms within the PFC during WM functions, it is reasonable to assume that dopamine may play a critical role in modulating rhythmic activity. For this reason, we studied the effects of dopaminergic D1 receptor activation on frequency dependent synaptic plasticity within different cortical layers. To study short term synaptic plasticity, stimulating electrodes were placed in either layer V or layer I to evoke excitatory post-synaptic potentials (EPSP). EPSPs were evoked in current clamp mode using an 8-pulse train stimulus (10-50 Hz train), followed by a 500ms recovery period; a single EPSP was evoked after the recovery period to monitor recovery. Initial experiments were performed in either the presence of APV (NMDA antagonist) or DNQX (AMPA antagonist) to isolate AMPA-mediated EPSPs or NMDA-mediated EPSPs, respectively. Protocols were then run in the presence of the D1 agonist, SKF38393, to determine the effects of D1 receptor activation on frequency dependent synaptic plasticity. Our results show that AMPA and NMDA-isolated responses show different forms of EPSP short-term dynamics (i.e. AMPA responses facilitate and NMDA responses depress irrespective of layer (I or V)). Additionally, we show that dopamine D1 receptor activation enhances AMPA EPSP amplitude with layer V stimulation, yet has no effect on AMPA EPSPs with layer I stimulation. Additionally, D1 receptor activation converted AMPA short-term dynamics from facilitating to depressing irrespective of layer stimulation. Similar to AMPA EPSPs, NMDA EPSP amplitude was also enhanced by D1 receptor activation with layer V stimulation. In contrast,

D1 receptor activation decreased NMDA EPSP amplitude with layer I stimulation. These results suggest that dopamine D1 receptor activation may alter signal processing differently for local processing (layer V) versus feedback processing (layer I). **Keywords:** Electrophysiology, Immunohistochemistry, Stereotaxic Injections, Retrograde Cell Labelling

54) Resistance or susceptibility to desensitization by presynaptic mu opioid and GABAB receptors is not due to differential effector coupling

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Presynaptic mu opioid (MOR) and GABAB (GABABR) receptors mediate inhibition of GABA release onto proopiomelanocortin (POMC) neurons of the arcuate nucleus. Presynaptic MORs are completely resistant to acute desensitization, while acute desensitization of presynaptic GABABRs is observed in approximately one quarter of recordings made from POMC neurons. It is currently unknown whether differential desensitization of presynaptic MORs and GABABRs can be accounted for by differential effector coupling. In the present study, whole-cell voltage clamp recordings were made from POMC neurons under conditions that disrupt several likely effectors of presynaptic MORs and GABABRs. MOR- and GABABR-mediated inhibition of GABA release was unaffected by blockers of voltage-dependent K+ channels and G-protein-coupled inwardly rectifying K+ channels. Inhibition of GABA release mediated by either receptor was also maintained in the absence of external Ca2+ or in the presence of ionomycin-mediated Ca2+ influx into presynaptic terminals. Together, these data suggest that neither the activation of voltage-dependent K+ channels nor inhibition of Ca2+ influx is required for MOR- or GABABR-mediated inhibition of GABA release to occur. However, inhibitory postsynaptic currents evoked in using an external solution containing Sr2+ in lieu of Ca2+ were still inhibited by both MORs and GABABRs. This result suggests that MOR- and GABABR-mediated inhibition of voltage-dependent Ca2+ channels does occur. Together with the observation that inhibition of GABA release mediated by MORs and GABABRs is not occluded by unregulated Ca2+ influx induced by ionomycin, it appears that inhibition of release by both types of receptors can be mediated by either the inhibition of Ca2+ influx or the by direct inhibition of vesicular release. These findings also demonstrate that MORs and GABABRs located on terminals presynaptic to POMC neurons are coupled similarly, and that differential receptor-effector coupling is unlikely to explain differential desensitization of presynaptic receptors.

55) Regulation of Synaptic Plasticity by Neuronal Lactic Acid Transport and Metabolism

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It is known that learning increases extracellular lactate levels and that lactic acid transport across neuronal membranes is required for learning and memory in normal subjects. Additionally, cognitive disorders including Alzheimer's disease, stroke, traumatic brain injury, and epilepsy involve perturbations in lactic acid metabolism. Despite this importance of lactic acid for learning and memory, hypotheses and evidence explaining the mechanism are sparse and controversial. For example, the astrocyte-to-neuron shuttle hypothesis proposes lactic acid release by astroglia supports mitochondria-dependent energy demands in neurons. In contrast, the neuron-to-astrocyte lactate shuttle hypothesis proposes neurons release lactic acid which is taken up by astrocytes to support their metabolism. Given the absence of mitochondria in dendritic spines, we reasoned lactic acid import might not facilitate changes in post-synaptic structure and function accompanying long-term-potentiation (LTP). Furthermore, since voltage gated calcium channels (VGCCs) are inhibited at lower intracellular pH, we hypothesized cytoplasmic acidification accompanying lactic acid uptake would inhibit VGCC-dependent LTP. Our pH and Ca2+ imaging experiments showed a dose-dependent decrease in cytoplasmic pH and bimodal modulation of voltage dependent Ca2+ influx in neurons exposed to lactate. Electrophysiological recordings showed VGCC-dependent LTP to be modulated by lactate with a dose response that mirrored the changes in Ca2+ influx. Lactate also modulated the effect that potentiating stimuli had on dendritic spine morphology. Combined our results suggest that, depending on concentration, lactate uptake into neurons can facilitate or inhibit learning through a mechanism involving changes in cytoplasmic pH. **Keywords:** Field potential recordings in slices, Intracellular pH and calcium imaging, Patch clamping, Cell culture

56) Mechanisms of Serotonin-2A Mediated Recurrent Oscillatory Bursting in Layer 5 Pyramidal Neurons of the mPFC

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We have recently reported that the serotonin-2A agonist TCB-2 elicits recurrent oscillatory bursting (ROB) in layer 5 pyramidal neurons of the medial prefrontal cortex of C57 mice. In short, in the presence of synaptic blockers (DNQX and gabazine) and TCB-2 pyramidal neurons will transition from tonic firing (3-4 Hz steady firing rate) to ROB discharge (bursts with mean firing rate of 26 Hz that recur at 1 Hz intervals) after 15-20 seconds of tonic stimulation. This bursting is similar to what has been reported following concurrent stimulation of the somas and apical dendrites of layer 5 pyramidal neurons which is dependent on the activation of L-type calcium channels of the apical dendrites. We are presently examining the ionic conductances that are modulated by TCB-2 to enable bursting. Our current model posits that serotonin mediated bursting results from the enhancement of a calcium-activated-nonspecific cation channel (CAN) and L-type calcium channels. Under this model, CAN activation is necessary to initiate bursting and L-type calcium channels of the apical dendrites drive individual bursts. Preliminary data indicate that this phenomenon is calcium-dependent as dialyzing cells with EGTA prevents ROB discharge. Future experiments will focus on CAN antagonism using flufenamic

acid, and antagonism of the L-type calcium channel via focal application of nimodipine to the neurons apical dendrites. **Keywords:** Serotonin, 5-HT2A, mPFC, Pyramidal Neuron

Neuroendocrine

57) Reproductive phenotype and GnRH system in Fgfr3-deficient mice

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The genesis of gonadotropin-releasing hormone (GnRH) system during development depends critically on fibroblast growth factor (FGF) 8 signaling through one of its cognate receptors, FGF receptor (FGFR) 1. Another cognate receptor, FGFR3, is not required for GnRH neuronal development, but it is unclear if FGFR3 participates in the maintenance of postnatal GnRH neurons and downstream reproductive function. The goal of this study is to characterize the postnatal GnRH system and reproductive phenotype of male and female transgenic mice harboring a null allele in FGFR3 (FGFR3+/- mice). Wildtype (WT) and FGFR3+/- mice were assessed for the timing of their pubertal onset, latency to first litter production, fecundity, and reproductive lifespan. Further, GnRH neuron numbers were determined for WT and transgenic mice between postnatal day (PN) 170-180. Results showed that FGFR3+/- males and females had significantly delayed puberty and truncated reproductive lifespan. FGFR3+/- females also exhibited a significant increase in latency to first litter production and a 30% reduction in fecundity. Lastly, although there was no significant difference in GnRH neuron numbers between WT and FGFR3+/- mice on PN170-180, the size of GnRH neuronal population was markedly variable in FGFR3+/- mice that have been housed with opposite sex, suggesting sexual interaction and FGFR3 deficiency interact to alter the size of adult GnRH neuronal population. In sum, FGFR3 deficiency leads to subfertile reproductive phenotype in both male and female mice. Further, this deficiency may alter the stability of GnRH neuronal population in mice with life-long exposure to opposite sex. (This work was supported by NIH R01 HD042634). **Keywords:** Fertility, GnRH neurons, FGF signaling, FGFR3, transgenic mouse, puberty,reproductive lifespan

58) Acute stress selectively and rapidly induces per1 expression in the medial prefrontal cortex, suprachiasmatic nucleus, and paraventricular nucleus of male and female rats

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The molecular clock is essential for the establishment of an organism's circadian rhythms. Disruptions to the expression of clock genes are associated with mood disorders (e.g., depression, anxiety). Recent studies have shown that acute stress increases per1 expression in mouse peripheral tissue and paraventricular nucleus of the hypothalamus (PVN), which is likely due to a glucocorticoid response element (GRE) located within the per1 promoter region. This stress effect has not been demonstrated in other brain regions. We hypothesize that stress may disrupt the molecular clock by altering per1 expression, leading to a diseased state (e.g., depression). Furthermore, females have a greater prevalence to certain mood disorders, as well as differences in stress reactivity compared to males. Thus, our objectives were to examine in male and female rats whether acute stress can alter clock gene expression in the suprachiasmatic nucleus (SCN), PVN, and prelimbic (PL) and infralimbic (IL) subregions of the medial prefrontal cortex (mPFC). The mPFC is important in stress reactivity and emotional regulation. Rats were maintained on a 12:12h light:dark cycle. Rats were challenged with 30 minutes restraint stress or taken directly from their home cage before immediately being sacrificed at zeitgeber time (ZT) 4 or 16. Plasma was obtained to measure glucocorticoid (CORT), ACTH, and estradiol levels. Brains were processed for use in in situ hybridization to quantify clock gene (per1, per2, bmal) and cfos mRNA. Acute stress rapidly induces per1, but not per2 or bmal, in the PVN, PL, and IL of both males and females. Despite females having greater stress-induced CORT, there were no sex differences in stress-induced clock gene expression. Stress-induced per1 was accompanied by stress-induced cfos expression in the PVN, PL, and IL. In the PL, males had significantly greater cfos compared to females. In the SCN, despite its lack of glucocorticoid receptors, stress also induced per1, but not per2 or bmal, expression in males, but only at ZT16, with a similar trend in females. Stress also induced cfos in both male and female SCN only at ZT16. These results suggest that acute stress selectively and rapidly increases per1 expression in the SCN (only at ZT16), PVN, and mPFC in both male and female rats. Per1 also contains a cAMP response element within its promoter region. The induction of per1 in the SCN, along with cfos induction, suggests that stress may alter per1 in the brain via CORT-independent, as well as CORT-dependent mechanisms. Supported by NIH grant: MH75968. **Keywords:** Clock genes, Sex differences, In situ hybridization

59) Reactive Oxygen Species Modulate Local L-type Calcium Channel Signaling in Gonadotropes

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The binding of hypothalamic neuropeptide gonadotropin-releasing hormone (GnRH) to its receptor on gonadotrope cells in the anterior pituitary initiates signaling cascades that result in the activation of extracellular signal regulated kinase (ERK) and subsequently enhanced luteinizing hormone biosynthesis. Previous work suggested that Ca2+ influx through L-type Ca2+ channels is necessary for ERK phosphorylation. More recently, we have directly visualized GnRH induced Ca2+ influx ("Ca2+ sparklets") mediated by L-type Ca2+ channels by using a combination of TIRF microscopy and

electrophysiology. We now want to examine molecular events that reside between the activation of the GnRHR and these biologically relevant localized subplasmalemmal Ca2+ signals. Reactive oxygen species (ROS) are recognized as cognate signaling molecules that regulate cell function. However, despite the generation of ROS being a ubiquitous phenomenon, ROS signaling has not been examined in gonadotropes. We hypothesize that ROS play a role in GnRH signaling and local L-type Ca2+ channel function. To test our hypothesis, we first determined if GnRH receptor stimulation increased ROS production. Using TIRF microscopy and a cell-permeant ROS indicator (2',7'-dichlorodihydrofluorescein diacetate (DCF); 1 μM) to monitor subplasmalemmal DCF fluorescence, acute stimulation of the GnRH receptor (with GnRH, 3 nM) produced localized ROS "puncta" in gonadotropes. If ROS contained in the puncta visualized near the plasma membrane modulate the activity of nearby L-type Ca2+ channels, then application of exogenous ROS should result in an increase in Ca2+ sparklets. Consistent with a stimulatory role, exposing gonadotrope cells to hydrogen peroxide (H2O2; 100 μM), a physiologically relevant ROS, increased local Ca2+ sparklet activity within 5 min (control nPs = 0.013 ± 0.005, H2O2 nPs = 0.30 ± 0.07; P < 0.05, n = 7). We next examined if endogenous ROS generators (e.g. NADPH oxidase) are involved with GnRH-dependent activation of L-type Ca2+ channels. To test the involvement of NADPH-derived ROS, we pharmacologically inhibited NADPH oxidase activity with apocynin (25 μM pretreatment for 5 min) followed by GnRH (3 nM). Apocynin pretreatment abolished stimulation of L-type Ca2+ channel sparklets by GnRH (P >0.05, n = 11). Cells treated with catalase (500U/mL), an enzyme to decompose intracellular H2O2, also decreased GnRH-induced Ca2+ sparklet activity compared to GnRH control (P >0.05, n = 6). Taken together, these data provide strong evidence that ROS signaling plays an important role in GnRH-dependent Ca2+ channel activity in gonadotropes since GnRH produced localized ROS generation, H2O2 was sufficient for stimulating localized Ca2+ influx, and inhibition of endogenous ROS generation decreased GnRH-induced Ca2+ influx. **Keywords:** TIRF microscopy, electrophysiology, calcium signaling

60) Dynamin is required for GnRH signaling to L-type calcium channels and activation of ERK

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We have shown that GnRH mediated engagement of the cytoskeleton induces cell movement and is necessary for GnRH signaling to ERK. It also has previously been established that a dominant negative form of the mechano-GTPase dynamin (K44A) attenuates GnRH activation of ERK. At present, it is not clear at what level these cellular events might be linked. To further explore this issue, we used a pharmacological inhibitor of dynamin GTPase activity, dynasore. Using the gonadotrope-derived αT3-1 cell line, we find that dynasore suppresses activation of ERK, but not JNK, following exposure to the GnRH agonist, buserelin (GnRHa). Live cell imaging displayed accumulation of dynamin-GFP in GnRH-induced lamellipodia and membrane protrusions. Coincident with translocation of dynamin-GFP to the plasma membrane, we demonstrated that dynamin colocalizes with the actin cytoskeleton and the actin binding protein, cortactin at the leading edge of the plasma membrane. Furthermore, exposure of αT3-1 cells to dynasore inhibited GnRH-induced cyto-architectural rearrangements. It has recently been discovered that Ca2+ influx via the L-type Ca2+ channels requires an intact cytoskeleton. Interestingly, not only does dynasore attenuate GnRH mediated actin reorganization, it also suppresses Ca2+ influx through L-type Ca2+ channels as visualized in living cells using total internal reflection fluorescence (TIRF) microscopy. Collectively, our data suggests that GnRH induced membrane remodeling events are mediated in part by the association of dynamin and cortactin engaging the actin cytoskeleton, that then regulates Ca2+ influx via L-type channels to facilitate ERK phosphorylation. **Keywords:** GnRH, ERK, Gonadotropes

61) Inhibition of organic cation transporter 3 (OCT3) in the central nucleus of the amygdala increases extracellular serotonin and reduces fear expression

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Organic cation transporter 3 (OCT3) is a corticosterone-sensitive low affinity high capacity transport protein, expressed in neurons and glial cells, that clears monoamines, including serotonin (5-hydroxytryptamine; 5-HT) from the extracellular space. The central nucleus of the amygdala (CE) has major reciprocal connections from monoaminergic nuclei such as the dorsal raphe nucleus and has been implicated in fear-related behaviors. Organic cation transporter 3 has been found to be expressed in the CE but little is known about its role in fear-related behaviors. We hypothesized that OCT3 modulates fear-related behavior by controlling extracellular 5-HT concentrations in the CE. We predicted that inhibition of OCT3, under basal or restraint stress conditions, would elevate extracellular 5-HT concentrations and inhibit fear as part of a negative feedback loop. We tested this hypothesis with two experiments. In Experiment 1 rats received unilateral reverse dialysis of one of two different OCT3 blockers, either corticosterone (CORT) or normetanephrine (NM), into the CE, superimposed with a 40 min period of restraint stress. We then analyzed extracellular 5-HT concentrations using high performance liquid chromatography with electrochemical detection. In Experiment 2 rats received bilateral microinfusions of vehicle, CORT or NM into the CE immediately before exposure to either home cage control conditions or restraint stress for 40 min. Subsequently rats were tested in the elevated plus-maze. Rats that received reverse dialysis of either CORT or NM superimposed with restraint stress, relative to reverse dialysis of vehicle, had significantly elevated levels of extracellular 5-HT measured using microdialysis. In addition, among rats exposed to home cage control conditions, but not among rats exposed to restraint, bilateral microinfusion of either CORT or NM into the CE decreased the duration of

freezing behavior in the elevated plus-maze, without affecting the percent time spent exploring the open arms. These findings suggest a significant role for OCT3 in the CE in control of serotonergic signaling and fear-related behaviors. **Keywords:** Serotonin, Amygdala, Fear

62) Functional evolution of gonadotropin-releasing hormone and adipokinetic hormone: Studies from the sea hare, Aplysia californica

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In vertebrates, gonadotropin-releasing hormone (GnRH) is required for reproductive activation and hence the propagation of species. Emerging evidence suggests that GnRH and several related peptides, including adipokinetic hormone (AKH), are members of the GnRH superfamily that arose around 680 million years ago. Although the reproductive role of GnRH is well established among vertebrates, such a role for other GnRH superfamily members remains controversial in protostomes. To gain insights into the biological function of protostomian GnRH homologs, we examined the expression pattern and biological activities of a GnRH (ap-GnRH) and AKH (ap-AKH) in a gastropod mollusk, Aplysia californica. To date, A. californica is the only known protostome that simultaneously expresses an endogenous GnRH and AKH and therefore represents an excellent model for examining the functional evolution of two related peptides in a single species. Localization of ap-AKH and ap-GnRH using in situ hybridization (ISH) and immunocytochemistry (ICC) revealed that they were produced in different regions of the central nervous system (CNS), with ap-GnRH produced by ganglia implicated in motor control, and ap-AKH by ganglia implicated in the homeostatic control of physiological functions. The in vivo effects of ap-GnRH and ap-AKH were consistent with their distribution patterns. Specifically, ap-GnRH injections triggered motor responses of the foot and parapodia, whereas ap-AKH injections triggered physiological responses such as reduced body mass and increased gut motility. Lastly, neither ap-AKH nor ap-GnRH acutely activated A. californica reproduction. These results support the hypothesis that the ancestral molecule that gave rise to GnRH and its homologs may be a more general neuroregulator responsible for diverse functions, and that reproductive activation may be a vertebrate-specific adaptation for GnRH signaling after the acquisition of the adenohypophysis.

63) Gonadotropin releasing hormone stimulates histone citrullination in gonadotrope cells

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Peptidylarginine deiminases (PAD) are a family of Ca2+ dependent enzymes that post-translational convert positively charged arginine into the neutral non-coded amino acid citrulline. Citrullination of histone tail arginine residues results in an open chromatin state causing changes in gene expression. Estrogen simulates expression of PAD isoforms 2 and 4 in the mouse uterus and mammary gland suggesting a possible role for PADs in reproduction. Since gonadotropin releasing hormone (GnRH) receptor signaling is also critical for fertility, we were curious if PADs are expressed in anterior pituitary gonadotropes. Currently, PAD isoform expression and the functional role of PAD catalyzed histone citrullination within gonadotropes remains unknown. To address this gap in knowledge, we first examined the LβT2 gonadotrope derived cell line for expression of different PAD isoforms. PAD2 mRNA levels were over 600 fold higher compared to the other PADs. To confirm our results in vivo, we found strong PAD2 expression throughout the human pituitary by immunohistochemistry. Next, we assessed the ability of the GnRH agonist Buseralin (GnRHa) to regulate PAD2 protein expression. LβT2 cells were treated with a time course of GnRH and western analysis of cellular lysates revealed that PAD2 levels increased rapidly with the highest expression seen at 30 minutes. Using an immunofluorescent approach, we observed punctate PAD2 staining in the nucleus of LβT2 cells after 30 minutes of GnRHa treatment. Given the upregulation and accumulation of PAD2 in the nucleus following GnRHa, we next examined if the functional consequence of this localization was to facilitate citrullination of histones. GnRHa treated LβT2 cells showed an increase in citrullination of histone H3 arginine residues 2, 8, and 17 in a time dependent manner. Collectively, we have found that GnRHa treatment of LβT2 cells results in increased PAD2 expression in the nucleus and citrullination of histones. We currently are identifying the genes that are regulated by PAD2 catalyzed histone citrullination in in gonadotropes in response to GnRH treatment. **Keywords:** Epigenetics, Gonadotropin releasing hormone, qPCR, Western Blots, Immunofluorescence

64) In a Salted State: High Salt Affects Metabolism and Puberty

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Humans are consuming an excessive amount of salt. There are known links between high sodium intake and the risk of stroke, hypertension, and heart attack. As it has been suggested that rat control diets are already too high in salt, we first fed Sprague-Dawley rats, (postnatal D21-45), either a low (0.01%), control (0.3%), or high (2.3%) salt diet. Rats were given the option of drinking tap water or saline (0.5%). Contrary to the earlier study, rats on low salt consumed the same amount of salt as rats on the control diet, with high salt rats consuming additional salt through drinking. A “western” diet is high in salt and fat. In a second study, Sprague-Dawley rats aged postnatal day 21-45 were fed control (0.3% salt, 10% kcal fat), high salt (HS; 8.3% salt, 10% kcal fat), high fat (HF; 0.3% salt, 60% kcal fat), or high salt + high fat (HS/HF; 8.3% salt, 60% kcal fat). Although HF animals ate more kcal/day initially, this difference disappeared after 4 days. Despite comparable consumptions, HF-low salt animals showed increased body, fat, heart and liver weight, as well as leptin levels. Both high fat diets excreted more lipids, but only HF-low salt rats produced more feces. Animals fed HS had higher kidney weights compared to low fat groups. HF did not affect puberty onset as estimated by vaginal opening. HS and HS/HF diets significantly delayed puberty onset. This delay remained even after providing elevated salt on alternate days.

Our results suggest that the control diet is not too high in salt, and rats, given the choice, will consume far greater quantities than current control diets offer. The HS/HF diet was significantly different from HF, suggesting that salt may play an dominant role in metabolism and puberty. **Keywords:** Neuroendocrinology, Reproduction, Obesity

65) Salt and Metabolism: A Dietary Investigation of Respiration and Lipid Output

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Processed foods containing high fat and sodium amounts are playing a crucial role in the world's obesity epidemic which is currently causing 3.4 million deaths per year. The average American ingests double the recommended amount of salt on a daily basis. If we are consuming such copious amounts, it needs to be understood how sodium affects metabolism. Sprague-Dawley rats postnatal day 21-45 were fed a control, high salt (HS), high fat (HF), or high fat/high salt (HF/HS) diet. Food intake, fecal output, fecal lipid output, respiratory quotient (RQ), activity, and internal temperature of these rats were measured. We report the HF diet fecal output was significantly higher than all other groups. The total lipid output of the HF was significantly higher than control and HS. Additionally, the total lipid output of the HF/HS was significantly higher than the control. There was an apparent trend with the mean control RQ 0.982 ±0.029, the HS 0.857 ±0.033, the HF 0.865 ±0.021 and the HF/HS 0.857 ±0.029. This suggests that control animals were metabolizing mostly carbohydrates and the other diet groups were metabolizing a combination of carbohydrates and lipids. It is interesting that both the HS populations had the lowest average RQs indicating they were metabolizing the most lipids. Preliminary results of activity and temperature show no significant differences. My research indicates that sodium affects mammals via their energy expenditure and energy storage, critical components of one's metabolism. Examining the differences of sodium and fat on lipid excretion and respiration in rats is progress towards understanding the specific effects of similar dietary variations in humans. **Keywords:** Metabolism, Sodium and Fat, Respiratory Quotient, Lipid Output, Locomotion, iButtons

66) Fetal Programming of Newborn Leukocyte Telomere Length

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The shortening of leukocyte telomere length is linked to increased disease risk and mortality, independent of chronological age. It is plausible that prenatal exposure to stress influences disease risk via telomere erosion, but little prospective work has explored the association between prenatal exposures and early telomere length. The current prospective study evaluates the association between maternal mood and anxiety late in gestation and newborn leukocyte telomere length in a prospective sample. Prenatal maternal mood and newborn telomere length were collected from 47 mother-infant dyads. Maternal anxiety (Beck Anxiety Index, BAI) and depression symptoms (Edinburgh Postnatal Depression Scale - EPDS) at ~29 gestational weeks were assessed. Dried blood spots were collected from newborns as part of a routine heel stick 24 hours after birth. Relative leukocyte telomere length (T/S ratio) was derived from newborn dried blood spots. Fetal exposure to elevated maternal anxiety at 29 gestational weeks was significantly associated with shortened leukocyte telomere length in healthy, full term newborns ($r(47) = -.29$; $p = .05$). Mothers who exhibited symptoms of anxiety had newborns with significantly shorter telomere length than those who were asymptomatic ($t(45) = 2.034$, $p = .048$). There was no significant association between maternal depression and leukocyte telomere length at birth ($r(47) = -.092$, $p = .54$). Fetal exposure to elevated maternal anxiety may contribute to early telomere erosion in leukocytes, as indicated by reduced telomere length at birth. These findings support the hypothesis that fetal exposure to maternal psychological stress may program long-term health and disease by contributing to the premature shortening of telomeres. Further research is required to understand the link between shortened leukocyte telomere length at birth and subsequent health and development. **Keywords:** Prenatal stress, birth outcomes, stress hormones

67) Glucocorticoid-dependent diurnal modulation of conditioned fear extinction and recall

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Post-traumatic stress disorder (PTSD) is characterized by abnormal prefrontal cortex (PFC) functioning and abnormal circadian parameters (e.g. glucocorticoid [CORT] circulation). PTSD patients also exhibit persistence of a generalized fear response in the absence of immediate threat. This persistence of fear could at least in part be due to the inability to successfully extinguish conditioned fear and/or recall extinction. Exposure therapy is one of the most widely used treatments for PTSD, and the aforementioned extinction and memory deficits are exemplified by the low success rate of this treatment paradigm. Even when within session therapy is successful, fear responses tend to reappear outside the therapeutic context in a phenomenon known as fear renewal. CORT has been shown in animal and human studies to modulate both emotional memory and circadian rhythm integrity. PTSD patients frequently exhibit blunted circadian CORT release. We have previously shown that the molecular clock is modulated by CORT circulation patterns in the PFC, a brain area that is integral to proper conditioned fear extinction and recall. Because optimal PFC neural function may be reliant on molecular clock operation, we hypothesized that conditioned fear extinction learning and renewal may vary with

time of day and the presence or absence of circadian CORT secretion. In these experiments we used a standard auditory conditioned fear protocol to train rats (n=6) to fear a tone (CS) that had been paired with a mild foot shock (US). 24 h later we presented rats with 15 trials of the tone alone to extinguish conditioned fear. 24 h later we tested rats for their ability to recall extinction. Either 72 hours (expt 1) or 24 hours (expt 2) later we put rats in a novel context and exposed them to 3 tones in order to measure fear renewal. Training and testing times were held constant and occurred either during the rat's active phase (Zeitgeber time [ZT] 16) or inactive phase (ZT 4). We found that conditioned fear extinction recall was enhanced and fear renewal was blunted in rats trained and tested at ZT16 compared to ZT4. These diurnal effects on extinction recall and renewal were absent in adrenalectomized (ADX) rats, with level of responses at both times of day comparable to those of sham-ADX rats trained and tested at ZT4. We conclude that the recall of extinction as well as fear renewal are affected by both ZT and CORT status, such that optimal recall is reliant on training/testing that occurs during the active phase an in the presence of circulating CORT. This study holds implications for optimizing PTSD treatment by targeting both the circadian system (i.e. diurnal CORT) and persistent fear. **Keywords:** Circadian rhythms, Prefrontal cortex, Conditioned fear, Extinction

Neuroscience Outreach/Education

68) Muscles Alive! A novel, experiential neuroscience education outreach program for elementary, secondary, and university students

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An effective strategy to improve neuroscience education is to make lessons fun, interactive, and immersive for students. Experiential, auditory, and visual modes of information transmission can optimize learning in diverse student groups. Our lab conducts experimental neuromuscular studies that often involve measuring the electromyogram (EMG) with expensive lab-based equipment not suitable for use in public education in the field setting. In 2012, a collaboration between our group at Colorado State University and the team at Backyard Brains (BYB), Inc., resulted in BYB's new Human EMG Spikerbox, a small, inexpensive bioamplifier that for the first time allows students of all ages to experience, hear, see, and record their own muscle electrical activity in school and community settings. The EMG signal is detected with 15-cent disposable electrodes made from common materials, displayed in real time via a free App on smartphones or tablets, and played through hobby speakers. The novel EMG kits are the centerpiece of our 1.5 yr-old program Muscles Alive!, named as an homage to the classic EMG text. Offering vivid visual and audio feedback, our participatory demonstrations include: 1) Live visual/auditory display of raw EMG signals from muscles of the hand, arm, leg, and face during different tasks; 2) Jaw muscle activity - chewing experiments with foods of different consistencies; 3) Weight lifting and arm wrestling ; 4) Occasional successful recording of single motor unit spike trains, allowing observation of single neuron discharge behavior; and 5) Tendon vibration demos with handheld massagers that target afferent muscle sensors: The Phantom Limb (biceps brachii tonic vibration reflex) and the Neurophysiology Trust Fall (body sway vibratory proprioceptive illusion). Our demonstrations, along with accompanying age-appropriate educational materials, are designed to teach students about 1) biological electricity and excitable cells, 2) the relation between brain command and muscle activation, 3) how action potentials are transmitted efferently to muscle and converted to force, 4) the role and extreme sensitivity of muscle sensors (spindles) in reflexes and proprioception, and 5) the essential role of voluntary muscle control in everything that makes us human. We have interacted with ~2,900 9-18 year-olds during > 40 events in public schools, science fairs, expos, and after school programs. The same equipment and concepts have been used successfully in large-audience settings with adults and we are currently implementing these techniques in university neuromuscular physiology and kinesiology courses. **Keywords:** Neuroscience, neuromuscular, education, outreach, public schools

Sensory and Motor Systems

69) Characterization of rubro-cerebellar reciprocal circuits

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The cerebellum is critical for motor coordination and learning. The cerebellar nuclei (CbN) are the sole output of the cerebellum and target, among other areas, the Red Nucleus (RN) to influence movement. Thus, understanding what drives CbN firing is critical to understanding how the cerebellum sculpts movement. Canonically it is thought that the only glutamatergic input to the CbN is from collaterals of inputs going to cerebellar cortex. However, RN has been reported to project to the CbN preferentially in the rat (Huisman et al., 1983). If so, it would suggest that the RN and CbN might form a reciprocal circuit. Here we used a combination of anatomical tracers and immunohistochemical markers to examine the organization of this pathway. First, we verified that a projection from the RN to CbN exists in the mouse after observing retrogradely labeled somata in the RN after Choleratoxin B injection into the CbN. We then used genetic and histological markers for both GABAergic and Glycinergic neurons to examine if CbN terminals contact inhibitory cells in the RN. Few if any inhibitory somata were identified in the RN, suggesting that the CbN targets the glutamatergic output cells and not inhibitory interneurons in the RN. Further, we have found that RN targets different cell types in the CbN, both inhibitory and possibly excitatory: Both BDA and AAV-labeled RN terminals contact glycinergic cell bodies and dendrites as well as other, unknown, cell types in the CbN. Taken together, this evidence suggests that CbN may be playing an excitatory role

to the RN, while RN inputs to the CbN might be more diverse. Understanding the anatomical characteristics of this reciprocal loop will lend insights to how this pathway may be contributing to cerebellar nuclear processing and ultimately motor output.

70) Characterization of AgRP Immunoreactivity in Taste Buds of C57 and ob/ob Mice

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Obesity is a growing epidemic which leads to multiple serious health consequences, and although the causes are complex, dysregulation of neuronal systems controlling appetite play an important role in overconsumption. In addition to changes in hypothalamic circuits, it now appears that changes in taste sensitivity occur. For example, leptin, which is released by lipid stores, binds to leptin receptors on taste cells and affects responses to sweet stimuli. (Maliphol et al., 2013; Niki et al., 2010). Our lab discovered that taste cells also contain Agouti-Related Peptide (AgRP), known to play a role in the hypothalamus regions involved in energy balance (Monahan et al., 2012). Based on this evidence, a working model of leptin-induced changes in taste sensitivity has been developed. Previous research evaluating this model indicated that AgRP immunoreactivity (ir) increases in taste buds of obese dB/dB mice lacking functional leptin receptors relative to taste buds from control mice (Pellow et al., 2013). In our current study, we further characterize AgRP expression in taste buds from both C57 and ob/ob mice lacking leptin. Using various markers for specific taste cell types combined with immunocytochemistry and confocal microscopy, we show that AgRP does not co-label with type II cells expressing PLCβ2 in C57 or ob/ob mice, nor does AgRP co-label with type III cells expressing serotonin. Interestingly, we show that AgRP-ir may be localized to gustatory nerve fibers surrounding type III cells in C57 mice. Double-labeling experiments with P2X2 antibodies showed that AgRP-ir fibers are not the gustatory fibers that express this ATP receptor. Based on these results, we conclude that AgRP is not likely expressed in type II cells, a subpopulation of type III cells, or in fibers expressing P2X2. Future experiments will focus on nerve fiber markers and alternate type III cell markers to more fully characterize the identity of the AgRP-ir cells in taste buds. In addition, we will also investigate the AgRP-ir pattern in obese mouse models to elucidate what role, if any, AgRP has in changes in taste sensitivity.

71) Development and Testing of an Electrotactile Tongue Stimulation Device for Sensory Substitution

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Hearing loss or impairment affects more than 30 million people in the United States according to the National Institute for Deafness and Other Communication Disorders. Congenital defects, disease, toxicity and noise-induced hearing loss all contribute to the prevalence of these disorders. Some of these individuals are greatly aided by cochlear implants, one of the most successful neural prosthesis to date (Moore and Shannon, 2009). Unfortunately, these are only effective for people with defects in the peripheral part of the auditory system since they stimulate the auditory nerve. Implants more centrally located in the auditory system are much less effective. Our group is developing a novel sensory substitution device for hearing loss, which uses patterns of electrical stimulation on an electrode array in contact with the surface of the tongue. To guarantee accurate transmission of audio information to the brain, each electrode on the array must depolarize an adequate number of somatosensory nerve endings to generate a noticeable sensation to the user. Additionally, the electrodes must be sized and spaced across the surface of the tongue such that two distinct sensations are felt when any two adjacent electrodes are simultaneously active. To design electrode arrays that meet these criteria, the effective nerve density of the surface of the tongue was mapped using randomized two point discrimination tests of electrical stimuli across a 4cm by 4cm area of the tongue. Participants placed a uniformly sized and spaced rectangular electrode array in their mouths and pressed their tongues against its surface. One or two electrodes were randomly activated and participants recorded the number of distinct points perceived in response to the stimulus. Additionally, they rated the intensity of the stimulus on a scale of one to ten. These parameters were mapped across the surface of the tongue, providing a rudimentary nerve density map that can be used to size and space electrodes on arrays used for sensory substitution. **Keywords:** Sensory Substitution, Electrotactile Stimulation, Auditory Prosthesis, Tongue.

72) Cellular and population analyses of signal filtering at olfactory bulb glomeruli

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GABAergic periglomerular (PG) cells in the olfactory bulb receive direct input from olfactory sensory neurons (OSNs) while also targeting glutamatergic external tufted (ET) cells that mediate feed-forward excitation of output mitral cells (MCs). It has been postulated that intraglomerular inhibition mediated by PG cells provides an alternate mechanism to lateral inhibition to decorrelate similar odors (Cleland & Linster, 2012). PG cells, which have a high input resistance (Puopolo & Belluzzi, 1998; Hayar et al., 2004), should be preferentially activated when a glomerulus receives weak OSN input (e.g., due to an “off-target” odor), resulting in inhibition of the glomerulus. Stronger inputs, in contrast, should excite excitatory elements at the glomerulus sufficient to overcome inhibition. In this study, we used patch-clamp and imaging approaches in rat olfactory bulb slices to assess this hypothesis. We first tested the assumption that PG cells are more responsive than ET cells to current input. Following electrical stimulation of OSNs, PG cells indeed required much smaller monosynaptic excitatory post-synaptic currents (EPSCs; 141±26 pA, n = 8) to generate action potentials than ET cells (297±36 pA, n = 16; p = 0.0093). At the same time, however, the relatively large dendritic arbor of ET cells resulted in

them having much larger EPSCs, which would favor ET cell excitation. During simultaneous pair-cell recordings (n = 4), the EPSCs in ET and PG cells, respectively, were 241 ± 134 pA and 45 ± 21 pA at a given stimulus intensity (4.5-10 μA). To test directly whether activation of PG cells or ET cells is favored at low levels of OSN activity, we used a population analysis of fura-2, AM associated calcium signals in VGAT-Venus transgenic rats, which selectively labels GABAergic cells. Weak OSN stimuli (5-20 μA) resulted in ~10 times more active presumed PG cells versus ET cells (ET to PG cell ratio = 0.09 ± 0.05, n = 8 glomeruli in 3 slices), although the relative number of active ET cells increased 3-fold (p = 0.0002) with stronger stimuli (10-50 μA) to a value (ET to PG cell ratio = 0.35 ± 0.05) that matched the total cell count ratio (ET to PG cell ratio = 0.37). Thus, the high input resistance of PG cells offsets their disadvantage of having a small number of OSN contacts, such that PG cells are mainly excited when a glomerulus receives weak input, but ET cell excitation catches up to PG cells with stronger input. These results support the hypothesis that the PG/ET cell microcircuit underlies a glomerular signal-filtering mechanism that could drive olfactory contrast enhancement. **Keywords:** Electrophysiology, Ca Imaging

Translational Neuroscience

73) Dietary queen bee acid reduces anxiety and stress-related weight loss in aged male rats.

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Royal jelly, a nutrient-rich solution that supports queen bee larvae in select honeycomb cells of a bee hive, has been used for centuries as a supplement because of suggestions that it may confer benefits for fertility, growth, skin quality and lifespan. Queen bee acid (QBA; 10-hydroxy-2-decenoic acid) is the predominant fatty acid (~2% w/w) in royal jelly, and QBA, specifically, induces the epigenetic effects on the honeybee genome that are associated with the larger growth and longer lifespan of the queen bee phenotype (Spannhoff et al. [2011] EMBO Rep 12:238). However, few data support brain-related QBA effects in mammals. We have performed two independent preclinical studies that sought to identify QBA effects in the rat brain. In the first study, male Sprague-Dawley rats were fed diets containing QBA (0-24 mg/kg bw/d), starting at 12 months of age. Dietary QBA administration continued while the animals were tested for exploratory activity (open field) at age 15 months, cognitive performance (Barnes maze) at age 16-17 months, anxiety-related behavior (elevated plus maze) at ages 15 and 17 months, and depressive-related behavior (forced swim test) at age 17 months. Body weight was also measured weekly throughout the study. QBA at a dose of 12 mg/kg/d was associated with faster learning on the Barnes maze, while the 24 mg/kg/d dose increased open field activity, decreased anxiety-related behaviors, and maintained body weight with age. A confirmation study that investigated QBA and its dietary combination with another nutrient, docosahexaenoic acid (DHA; 1% of total fatty acids), was also performed. In this second study, the QBA effects on anxiety (12 and 24 mg/kg/d) and weight maintenance (24 mg/kg/d) were confirmed. In addition, DHA sufficiency reduced anxiety and maintained weight over time to a similar extent as QBA. However, QBA was necessary to mitigate the weight loss that was associated with the stress of the behavioral studies, and DHA alone had no effect on this type of weight loss. These findings suggest that QBA and DHA have similar effects on anxiety-related behavior, but the two ingredients may have interactions in the stress-related physiology of aged animals. Thus, the combination of QBA with DHA may be an ideal approach for reducing anxiety-related phenotypes through the diet. Future experiments should address whether these same dietary manipulations affect behavior or weight maintenance in earlier age ranges.

74) Dietary N-methylserotonin from Japanese pepper (Zanthoxylum piperitum) regulates skin temperature in a female rat model of menopause-related hot flash

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Clinical evidence suggests that supplementation with black cohosh (Cimicifuga racemosa) relieves symptoms of menopause, but the data are mixed. N-methylserotonin (NMS) is a minor component of black cohosh and is a selective agonist of a serotonin (5-HT) receptor subtype that is involved in thermoregulation (5-HT7). NMS also has inhibitory activity at the serotonin reuptake transporter [SERT; Powell et al. (2008) J Agric Food Chem 56:11718-26]. These findings implicate NMS as an active component of black cohosh that may relieve hot flash and mood-related symptoms of menopause, but the in vivo effects of NMS are not established. We performed two studies that sought to determine the effects of dietary supplementation with NMS on induced hot flash and mood in female rats. In the first study, ovariectomized (OVX) female rats were fed diets that contained different levels of NMS or were given estradiol implants. The animals were then tested for locomotor activity (open field), anxiety-like behaviors (elevated plus maze), and depression-like behaviors (forced swim test). Hot flashes were subsequently induced with intravenous calcitonin gene-related peptide, and skin temperature was monitored. NMS supplementation did not affect OVX-induced weight gain, uterine growth, or mood-related behaviors. However, NMS supplementation significantly blunted the hot flash response in a manner similar to that observed in estradiol implanted animals. In the second study we used Japanese pepper (Zanthoxylum piperitum) as an NMS source because it contains ~25-fold more NMS than black cohosh. This study confirmed the results of the first study and determined that dietary supplementation with a natural source of NMS was just as effective as synthetic NMS in reducing the hot flash response. These in vivo findings support NMS as an active

component of black cohosh and Japanese pepper that could decrease menopausal hot flashes without the potential risks associated with hormone replacement therapy or phytoestrogen use.