11th Annual Meeting of the Front Range Neuroscience Group

December 4, 2013
Hilton Fort Collins
11th Annual Meeting: December 4, 2013
Hilton Fort Collins
Registration 10am; Program 10:30am – 6:30pm

10:30-11:30 – Bryan Dik, PhD Colorado State University
"Forging a Career Path that Fits"

11:30-noon – Special Guest Speaker on Creativity
Bruce Adolphe: Composer, Author, Innovator
“The Mind’s Ear – Dreaming and Thinking Music”

Noon-3pm – Lunch, Posters, Vendors!
1:00--2:00 ODD
2:00--3:00 EVEN

3-4pm – Award Winning Student presentations
Matt Dicken (CSU)
Peter Grace (Univ Colorado Boulder)
Joseph Zak (CU Anschutz)
Ryan Tooker (CSU)

4:00-4:30pm – Coffee break, stretch

4:30-5:30pm – SfN Distinguished Traveling Scientist Keynote:

Stephen J. Smith, PhD
Stanford University School of Medicine

Synapse Diversity:
Why It Matters and How To Measure It

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

http://FRNG.colostate.edu
Morning Session: "Forging a Career Path that Fits"

Bryan Dik, PhD, is an associate professor of psychology at Colorado State University and co-founder and Chief Science Officer of jobZology. Dr. Dik received his degrees from Calvin College (BA, 1998) and University of Minnesota (PhD, 2005). His research is primarily in the area of career development, especially perceptions of work as a calling; meaning, purpose, religion and spirituality in career decision-making and planning; measurement of vocational interests; and career development interventions. He has published more than 60 journal articles and chapters, is co-author of "Make Your Job a Calling: How the Psychology of Vocation Can Change Your Life at Work" (2012, Templeton Press), and is co-editor of "Psychology of Religion and Workplace Spirituality" (2012, Information Age Publishing) and "Purpose and Meaning in the Workplace" (2013, APA Books). He serves on the editorial boards of six research journals, including Journal of Counseling Psychology, Journal of Vocational Behavior, and Journal of Career Assessment. He is recipient of the 2010 Early Career Professional Award from the Society for Vocational Psychology.

jobZology is a start-up company (spun out from CSU) that assesses and provides feedback to job-seekers and facilitates matches to employers based on psychological fit. Its technology disrupts the traditional inefficient methods of job seekers finding work, replacing antiquated processes and methods with an application interface that enables a scientific match between job seekers and employers. The software helps job seekers understand more about themselves and their key drivers to meaningful work, and matches them to organizations where they will thrive – essentially an "eHarmony for jobseekers and employers."
Special Guest Speaker

Bruce Adolfe
Title: “The Mind’s Ear – Dreaming and Thinking Music”

The Mind's Ear offers a unique approach to stimulating the musical imagination and inspiring creativity, as well as providing detailed exercises aimed at improving the ability to read and imagine music in silence, in the "mind's ear."

SfN Distinguished Traveling Scientist Lecturer:

Stephen J. Smith, PhD
Prof. of Molecular and Cellular Physiology
Stanford University School of Medicine

Synapse Diversity: Why It Matters and How To Measure It

Research in the Smith Laboratory has focused on mechanisms of neural circuit development and function. Most of this work has involved devising new electrophysiological and optical methods then using those new methods to open new areas to exploration. Two areas of particular recent interest are imaging at diverse levels to visualize both connectome and synaptome in brain.
**Acknowledgements:**
Cover Page: Designed by Christina Dennison
Scientific images provided by Matt Dicken (CSU), Peter Grace (Univ Colorado Boulder), Joseph Zak (UC Health) and Ryan Tooker (CSU). Details will be in their oral presentations. The FRNG website (http://FRNG.colostate.edu) was created by Leif Saul in 2005 – see more images on our website. Thanks again to Leif for creating our electronic abstract submission system!!

Special Thanks!

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

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

Special thanks to our Platinum Level Industry Supporters: DSM Nutritional Products and Olympus America. In addition, special thanks to the Hilton Fort Collins for stepping up to a platinum level of support in providing the ideal venue and extra contributions, and to Jessica Veal in particular for help in making this all possible.

Special thanks to the University departments and programs that provided financial support to help make the meeting possible; in particular Colorado State University, the University of Wyoming, the University of Colorado at Boulder and finally the parent Society for Neuroscience.

Special thanks to the graduate student organizing committee for creating and polishing the program and fixing the details, and in particular for creating the program book. This includes Krystle Frahm, Christina Dennison, and Mallory Shields from CSU, Dori R. Pitynski and Colleen M. Cassidy from Univ Wyoming, John Soltys and Tara Martin from UC Anschutz Health Science Campus, Elizabeth Woodruff and Phil Siebler from UC-Boulder and Josie Gray 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, Scott Barbee, Mark Basham, Sondra Bland, Mark Thomas and Stuart Tobet.
BRAIN AWARENESS WEEK 2014!!!
Neuroscience Outreach Program at Ft Collins High Schools:

Spread the word, we need more scientists.
Open to faculty, postdoctoral fellows, graduate students, undergraduate students, professional research associates, research scientists, staff and those affiliated with neurobiology that have an interest in teaching neuroscience and research methods to teens.

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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. Inhibition in neural circuits involved in energy balance regulation.
   Matthew S. Dicken, Shane T. Hentges. From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.

2. Therapeutic morphine prolongs neuropathic pain in Fischer 344 rats: a role for TLR4 and inflammasome signaling in the lumbar spinal cord.
   Peter M. Grace, Keith A. Strand, Erika L. Galer, Yingning Zhang, Lisa I. Greene, Kenner C. Rice, Steven F. Maier, Linda R. Watkins. From the Department of Psychology and the Center for Neuroscience, University of Colorado, Boulder, Colorado; Chemical Biology Research Branch, National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism, Rockville, Maryland.

3. Control of glomerular output by metabotropic glutamate receptors in the olfactory bulb.
   Joseph D. Zak and Nathan E. Schoppa. From the Department of Physiology and Biophysics, University of Colorado School of Medicine, Aurora, CO.

4. Nitric Oxide Mediates Plasticity of Retinal Bipolar Cell Output.
   Ryan Tooker, M. Lipin, V. Leuranguer, E. Rozsa, J. Bramley, J. Harding, M. Reynolds & J. Vigh. From the Department of Biomedical Sciences, College of Veterinary Medicine and Biomedical Sciences; Dept. of Chemistry, College of Natural Sciences, Colorado State University, Ft. Collins, CO.

ORAL ABSTRACTS

Matthew S. Dicken, Shane T. Hentges.

Inhibition in neural circuits involved in energy balance regulation.
Proopiomelanocortin (POMC) and neuropeptide Y / Agouti-related peptide (NPY/AgRP) neurons have been studied extensively for their opposing regulation of food intake and energy homeostasis. Much interest has been recently focused on the effects of the amino acid (AA) transmitter GABA, which is released by both POMC and NPY/AgRP cell populations. It is unknown how the same inhibitory neurotransmitter is utilized by opposing cell types in order to reliably maintain energy balance. To address this issue, an overnight fast was used as an independent variable to push an animal to an energy deficit, presumably changing the physiology of both the orexigenic NPY/AgRP and anorexigenic POMC cells. Using optogenetic and electrophysiological techniques, the present study follows a reductive approach and aims to elucidate presynaptic changes in GABA release specifically in NPY/AgRP cell inputs caused by fasting. Conveniently, many NPY/AgRP neuron projections terminate onto POMC neurons, providing a downstream target from which electrophysiological recordings can be performed. While recording from POMC cells in mouse brain slices, GABA was light-evoked from AgRP cells expressing the light-sensitive cation channel Channelrhodopsin-2. By comparing the paired-pulse ratios of fasted and fed animals, it was determined that the probability of release from NPY/AgRP terminals is increased in animals that have not had access to food for 16 hours. Using a vesicle depletion protocol, an increased readily releasable pool was calculated in NPY/AgRP cells when animals were fasted overnight as compared to the control fed state. In situ hybridization for Gad67, the enzyme responsible for catalyzing the synthesis of GABA from glutamate, was used to corroborate electrophysiological findings; an increased number of NPY/AgRP cells colocalized with the Gad67 probe in fasted animals. Interestingly, Gad67 expression is reduced in POMC cells when mice are fasted. The inverse relationship of Gad expression in these two opposing neuron populations suggests a differential regulation of release, offering an explanation to why cells that have opposite functions from each other are using the same AA transmitter to regulate their downstream targets.
Therapeutic morphine prolongs neuropathic pain in Fischer 344 rats: a role for TLR4 and inflammasome signaling in the lumbar spinal cord

Despite their status as gold-standard therapeutic analgesics for neuropathic pain, opioids initiate microgliosis and proinflammatory cytokine release via Toll Like Receptor 4 (TLR4) in naïve animals, which enhances nociceptive neuroexcitability. 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 an inflammasome-dependent fashion involving TLR4, and the purinergic receptor P2X7 and caspase-1, which facilitate release of mature interleukin (IL)-1β.

Beginning 10 days after CCI surgery in male Fischer 344 rats, s.c. morphine (5 mg/kg b.i.d.) or equimolar saline was administered for 5 consecutive days. Compared to vehicle, morphine treatment significantly prolonged the duration of CCI-induced allodynia in F344 rats from 5 to 11 weeks (n=6/group; p<0.05). Continuous intrathecal infusion of inhibitors of TLR4 ([+] -naloxyone; 60 µg/h), P2X7 (Brilliant Blue G; 30 ng/h), or caspase-1 (ac-YVAD-cmk; 1 µg/h) prevented morphine-prolonged CCI-allodynia when administered concomitantly with morphine, and completely reversed established morphine-prolonged CCI-allodynia when administered 5 weeks after morphine dosing (n=6/group; p<0.05). Morphine treatment induced significant release of tumor necrosis factor (TNF)α and IL-1β (p<0.05) from lumbar dorsal spinal cords (p<0.05).

Furthermore, a single intrathecal dose of soluble TNF receptor or interleukin-1 (IL-1) receptor antagonist (100 µg; Amgen) transiently attenuated morphine-prolonged CCI-allodynia (n=6/group; p<0.05). Finally, to determine the role of sustained microgliosis in maintaining morphine-prolonged CCI-allodynia when administered 5 weeks after morphine dosing (n=6/group; p<0.05). Morphine treatment induced significant release of tumor necrosis factor (TNF)α and IL-1β (p<0.05) from lumbar dorsal spinal cords (p<0.05).

Control of glomerular output by metabotropic glutamate receptors in the olfactory bulb.

Olfactory contrast enhancement is often proposed to arise through GABAergic inhibitory mechanisms that filter odor signals based on their strength. Here we examined a novel mechanism that could underlie such signal filtering within olfactory bulb glomeruli that depends on the commonly-observed ability of metabotropic glutamate receptors (mGlurRs) to down-regulate GABA release (see e.g. Hayashi et al., 1993). Because mGlurRs are likely to be mainly activated by extrasynaptic glutamate, their activation should occur when a glomerulus receives strong inputs from olfactory sensory neurons (OSNs); hence disinhibition and passage of excitatory signals should preferentially happen with strong inputs. Importantly, it has also been established that extrasynaptic glutamatergic signaling is robust in glomeruli. To test the role of mGlurRs in glomerular signal processing, we performed patch-clamp recordings in rat olfactory bulb slices, first asking what effect mGlur activation has on inhibition from GABAergic periglomerular (PG) cells onto tufted cells, a critical regulator of activation of output mitral cells. We found that local puff-application of the Group II mGlur-specific agonist DCG-IV (5 µM) on a glomerulus profoundly reduced GABA release from PG cells onto tufted cells evoked by OSN stimulation (47±15% decrease in inhibitory current, n=5; p = 0.035), while also increasing the number of evoked action potentials in tufted cells (66±20% increase, n=6; p = 0.023). At the same time, an antagonist for Group II mGlurS (LY341495) enhanced GABA release from PG cells (25±5% increase in inhibitory current, n = 6; p = 0.005). Thus, activation of mGlurS in glomeruli can disinhibit tufted cells through modulation of GABA release, and moreover, these receptors can be activated by native glutamate transients. In terms of the mitral cell response, we found complex, biphasic effects of mGlur activation. Puff-application of the agonist DCG-IV hyperpolarized mitral cells (1.1±0.2 mV, n = 9) and reduced their spiking due to OSN stimulation (64.4±17.5% decrease, n = 5; p = 0.021), different from the enhanced excitation seen in tufted cells, but there was depolarization and enhancement in spiking above control levels upon immediate removal of DCG-IV (in 5 of 5 cells). The enhanced excitation of mitral cells following the mGlur-mediated hyperpolarization could be due to hyperpolarization-activated cation channels. Our results suggest that mechanisms exist to support an
mGluR-dependent glomerular signal filter, although the temporal dynamics of the filter could differ between the output tufted cells and mitral cells.

Ryan E Tooker, M. Lipin, V. Leuranguer, E. Rozsa, J. Bramley, J. Harding, M. Reynolds and J. Vigh

Nitric Oxide Mediates Plasticity of Retinal Bipolar Cell Output

The retina adapts to large, daily changes in natural light intensity by shifting its dynamic range of coding. For example, as morning light intensity increases, the retina implements multiple strategies that result in decreases in overall sensitivity in order to avoid saturation. However, adaptation to bright environments poses the inherent risk of losing visual information carried by dim/weak signals in complex natural scenes. Here, we report a novel form of activity-dependent plasticity in the retina which may serve to resolve this problem. In goldfish retinal slice preparation, strong depolarization of Mb type bipolar cell (BC) terminals lowered the threshold for calcium spike initiation by shifting the activation of voltage-gated calcium currents (I_{Ca}) to more negative potentials. The process depended upon glutamate-evoked retrograde nitric oxide (NO) signaling as it was eliminated by ionotropic glutamate receptor blockers or by an NO synthase blocker, TRIM. The NO-dependent I_{Ca} modulation could be blocked by N-Ethylmaleimide (NEM), indicating that NO acted via an S-nitrosylation mechanism. Importantly, the NO action resulted in a “weighted potentiation” of Mb output in response to small (≤-30 mV), but not large depolarizations. Further, light flashes with intensity ≥2.4x10⁸ photons/cm²/s were sufficient to “sensitize” the responses of Mb terminals to consecutive dim, rod-mediated inputs in a NEM-sensitive manner but did not alter bright, cone-mediated responses. Sensitizing light stimulation, capable of potentiating rod-mediated responses, induced increased S-nitrosylated protein immunolabeling rather selectively at locations closely associated with Mb terminals in goldfish retinas. Importantly, sensitizing light stimulation triggered S-nitrosylation immunolabeling pattern in mouse retinas similar to that seen in goldfish. Further, there was a drastic reduction in overall S-nitrosylated protein immunoreactivity in light adapted retinas from mice lacking neuronal NO synthase. We propose that this novel form of activity dependent, NO-mediated potentiation of rod-dominated BC output selectively increases the gain of dim retinal inputs at the BC output. In the healthy retina this process is well positioned to counteract decreases in sensitivity during light adaptation by preventing the loss of critical visual information carried by dim signals. Future studies will determine if the described mechanism contributes to elevated retinal glutamate levels and excitotoxicity in diabetic or ischemic retinal neuropathy where elevated retinal NO levels were reported.
POSTER PRESENTATIONS

Cognition and Behavior

1. Isolation rearing attenuates social encounter-induced c-fos in the rat forebrain. SONDRA T BLAND, MAhern, D Goodell, S Grotewold

2. Voluntary exercise during fear extinction reduces fear renewal: Role for activation of reward circuitry during extinction. COURTNEY A BOUCHET, AMika, KG Spence, JE Hellwinkel, S Campeau, HEW Day, M Fleshner, BN Greenwood.

3. Common Statistical Errors in Experimental Neuroscience Studies. WAYNE BRINER.

4. High-Fat Diet Attenuates Motivated Behavior. PAIGE M. DINGESS, BJ Anderson, RA Darling, TE Brown


6. Acute and repeated social defeat differentially activate hypothalamic orexin/hypocretin and melanin-concentrating hormone neurons in the male rat. ED Paul, BRANDON KK FIELDS, JD Heinze, MW Hale, JL Lukkes, IA Kerman, CA Lowry.


9. Effects of menstrual cycle phase and oral contraceptive use on perception of emotional stimuli with reproductive significance: An event-related potential study. POLINA A. REYNOLDS, MA Kisley.

10. Guppies show significant levels of repeatability in many but not all exhibited behaviors. SEAN P STREICH, E Fischer, K Hoke


Development


15. A developmentally compromised serotonergic system is associated with reduced serotonin neuronal activation in response to stress: the role of fibroblast growth factor 8. LEAH R BROOKS, RA Woolaver, CL Enix, CA Lowry, PS Tsai.


17. Pericyte morphology differs by brain region in mice: Focus on the paraventricular nucleus of the hypothalamus. KRISTLE A FRAHM, SA Tobet.
18. The persistence of exercise-induced stress resistance depends on the developmental stage during which exercise is initiated. AGNIESZKA MIKA, CA Bouchet, KG Spence, BN Greenwood, M Flesher.

19. Impacts on input and output neurons of the suprachiasmatic nuclei in fibroblast growth factor 8 deficient mice. ANNIE V. MILLER, SI Kavanaugh, A Sanchez, SA Christopher, MA Basse, SD King, P-S Tsai.


**Disorders of the Nervous System**

22. A potential compensatory role for endogenous striatal tyrosine hydroxylase-positive neurons in a non-human primate model of Parkinson’s disease. ANDREW N BUBAK, DE Redmond, Jr., JD Elsworth, RH Roth, TJ Collier, KB Bjugstad, BC Blanchard, JR Sladek, Jr.

23. Inhibition of hypothalamic POMC neurons increases food intake in an activity-based anorexia mouse model. CHRISTINA S DENNISON, ST Hentges.

24. Brain metabolomics of rapamycin treatment in the Ts65Dn mouse model of premature aging and Down syndrome. NATHAN DUVAL, GN Vacano, D Patterson.

25. MicroRNA expression during differentiation of embryonic stem cells to midbrain dopamine neurons. MICHAEL FERREYROS, YM Lee, CR Freed

26. A novel peptide inhibits TRPM2 channel activation and reduces infarct size after middle cerebral artery occlusion. ANNA K GARSKE, T Shimizu, RJ Traystman, PS Herson

27. TAR DNA-binding protein-43 localization and function are coupled to mitochondrial physiology. JOSIE J. GRAY, HM Wilkins, DA Linseman.

28. The Nurr1 ligand 1,1-bis(3′-indolyl)-1-(p-chlorophenyl) methane (C-DIM12) supports a dopaminergic phenotype in multiple neuronal cell lines. SEAN L HAMMOND, S Safe, R Tjalkens.

29. Exploring the role of the nucleus tractus solitarius proopiomelanocortin neurons in the regulation of energy balance. ALEXANDER R HUGHES, ST Hentges


31. A novel anti-inflammatory diindolylmethane activator of NR4A2/Nurr1 is neuroprotective in a mouse model of Parkinson’s disease. BRIANA DE MIRANDA, KA Popichak, S Safe, RB Tjalkens

32. Activation of the nuclear receptor Nur77 by a novel diindolylmethane analog suppresses inflammatory gene expression in astrocytes. KATRIANA A POPICHAK, BR De Miranda, DL Carbone, S Safe, RB Tjalkens.

33. Mice with genetic deficiency for complement receptor type 2 (CR2) show neuroprotection after experimental closed head injury. MEGAN C RICH, MD Neher, CN Keene, S Weckbach, AL Bolden, JT Losacco, VM Holers, PF Stahel.

34. Dysregulation of Rac of Rho induces death of motor neurons and activation of these GTPases is altered in the G93A mutant SOD1 mouse model of ALS. TRISHA R STANKIEWICZ, RJ Bouchard, DA Linseman.


**Neural Excitability, Synapse and Glia**

40. Single particle tracking of Nav1.6 molecules demonstrates different mechanisms for sodium channel anchoring within the AIS versus the soma of hippocampal neurons. ELIZABETH J AKIN, K Brown, AV Weigel, S Sadegh, J Higgins, D Krapf, MM Tamkun.

41. A casein kinase 1 delta mutation in familial migraine with aura and sleep disorder. EMILY BATES, KC Brennan, R Shapiro, A Charles, L Ptacek.

42. Wheel running produces widespread structural changes in striatal and limbic regions involved in stress. PR Ghasem, A Mika, EA Sisernos, MA Keag, SM Engel, DANIEL BORCHERT, WC Craig, SJ Bowers, PJ Clark, BN Greenwood, M Fleschner

43. Optical detection of glutamate transients within olfactory bulb glomeruli. JENNIFER N BOURNE, JD Zak, CG Dulla, NE Schoppa.

44. Mitochondrial Control of Angiotensin Signaling in Cerebral Vascular Smooth Muscle. NATHAN L CHAPLIN, AM Fresquez, GC Amberg.

45. A Large Field-of-View Nonlinear Microscope for Biological Imaging. JEFFREY J FIELD, MD Young, CM Eitel, SA Tobet, JA Squier, RA Bartels.

46. Endoplasmic reticulum-plasma membrane contacts function as membrane protein trafficking hubs at the neuronal soma. PHILIP D FOX, CJ Haberkorn, AV Wiegel, EJ Akin, MJ Kennedy, D Krapf, MM Tamkun.

47. Aβ42 Induces Neuronal Hyperexcitability in a Pathway that Leads to Increased Seizure Sensitivity, Neurodegeneration, Motor Deficits, and a Shorter Lifespan. Y Ping, EU-TEUM HAHM, GIRMA WARO, D Vo-Ba, and S Tsunoda

48. Effects of dopamine on frequency dependent synaptic transmission. JONNA M. LEYRER, MP Thomas

49. Mu-opioid and GABAB receptor mediated inhibition of GABA release onto POMC neurons occurs through a mechanism that is Ca2+-independent and resistant to desensitization. REAGAN L. PENNOCK, ST Hentges

50. The Role of the C2A Domain of Synaptotagmin in Asynchronous Release. MALLORY SHIELDS, N Reist

51. Activation of serotonin-2a receptors induces rhythmic oscillatory bursting in layer 5 pyramidal neurons of the mouse medial prefrontal cortex. MICHAEL S SPINDLE, MP Thomas
52. Effects of repeated voluntary or forced exercise on rat brain thermosensitive serotonergic systems. MATT ARNOLD, BN Greenwood, JA McArthur, PJ Clark, M Fleshner, CA Lowry

53. The presence of endogenous glucocorticoids differentially influences acute stress-induced clock gene expression in extra-SCN regions of the rat brain. LAUREN E CHUN, JA Christensen, LR Woodruff, LR Hinds, RL Spencer

54. Actin cytoskeleton modulates local L-type calcium channel signaling and ERK activation in gonadotropes. AN K. DANG, Di Murtazina, C Magee, AM Navratil, CM Clay, GC Amberg

55. Chronic glucocorticoid intake alters basal Tph2 protein expression in anxiety- and resilience-related midbrain serotonergic systems. NINA C DONNER, AJ Matti, CA Lowry.

56. Identifying mechanisms by which exercise prevents inescapable stress induced instrumental learning deficits. PJ Clark, J Amat, PARS R. GHASEM, SO McConnell, SF Maier, BN Greenwood, M Fleshner.

57. Glucocorticoid-dependent dynamic modulation of sgk1 gene expression within oligodendrocyte dense white matter of rat brain in response to acute stress and time of day. LAURA HINDS, JA Christensen, LE Chun, L Woodruff, RL Spencer.

58. Localization and physiological characterization of adipokinetic hormone (AKH) in the sea hare, Aplysia californica. JOSHUA I JOHNSON, SI Kavanaugh, PS Tsai.

59. ICV infusion of (pro)renin receptor antagonist (mPRO20) attenuates prorenin and DOCA-salt induced hypertension. WENCHENG LI, Yumei Feng.


61. Altered serotonergic gene expression associated with anxiety-like behavior after Crh receptor priming in the bed nucleus of the stria terminalis. SOFIA MANI, NC Donner, SD Fitz, A Shekhar, CA Lowry.


63. Similar clock gene mRNA rhythmic expression in both male and female rats in medial prefrontal cortex. SARAH J MORTON, LE Chun, LR Woodruff, LR Hinds, RL Spencer.

64. New Tool to Modulate Levels of Endogenous Testosterone in Adult Male Songbirds. KARAGH MURPHY, S Slaugh, M Burton, D Wilson, J Dunning, K Carlson, JF Prather.

65. Roles of signaling pathways in the development and maintenance of the GnRH neuronal system. CONNOR NASH, M Ahow, M Pampillo, A Babwah, S Tobet.


68. Dietary N-methylserotonin regulates skin temperature in a female rat model of menopause-related hot flash. MICHAEL J WEISER, CM Butt.
69. The timing of Corticosterone peak circulation affects rhythmic clock gene expression in the rat prefrontal cortex. LIZ WOODRUFF, LE Chun, LR Hinds, RL Spencer

**Sensory and Motor Systems**

70. The acoustical cues to sound location in the adult Guinea pig: measurements of Directional Transfer Functions (DTFs). KELSEY ANBUHL, AT Ferber, K Koka, W Williams, JL Thornton, DJ Tollin.

71. The dependence of the binaural interaction component (BIC) of the auditory brainstem response (ABR) on binaural cues to sound source location in the guinea pig (Cavia porcellus). ALEXANDER T FERBER, NT Greene, KL Anbuhl, DJ Tollin.

72. Audibility Effects on Cortical Processing. HANNAH GLICK, L DURKEE, J Campbell, A Sharma

73. Spatial hearing capabilities of the adult guinea pig (Cavia porcellus). NATHANIEL T GREENE, AT Ferber, KL Anbuhl, PD Allen, DJ Tollin.


76. Cholinergic Neurotransmission Links Solitary Chemosensory Cells to the Immune System. CECIL J SAUNDERS, M Christensen, TE Finger, M Tizzano.

77. Transgenic expression of endogenous calcium indicator GCaMP3 allows visualization of somatic and visceral sensory neurons In vivo. BM Davis, J DeBerry, KRISTEN M SMITH, CJ Woodbury.

78. Control of glomerular output by metabotropic glutamate receptors in the olfactory bulb. JOSEPH D. ZAK, NE Schoppa

**Art**

79a. Emily Bates, “Migraine”

79b. Dori Pitynski, “Neuroconstellations”

80a. Jake Saunders, Untitled

80b. Aimee Winter, “A Winter Neuroscience Composition”
ABSTRACTS

Cognition and Behavior

1. Isolation rearing attenuates social encounter-induced c-fos in the rat forebrain.
   SONDRA T BLAND, M Ahern, D Goodell, S Grotewold. From the Departments of Psychology and Biology, University of Colorado Denver, Denver, CO.

Social deprivation during adolescence, known as isolation rearing, produces long-lasting changes in behavior and brain function in rats. We have previously reported that isolation rearing attenuated the increase in c-fos expression in the medial prefrontal cortex (mPFC) that was observed in group-reared rats after a brief social experience with a novel same-sex rat. Here we investigate the effects of isolation rearing on social experience-induced c-fos in other forebrain regions. Male and female Sprague-Dawley rats were purchased at postnatal day (P) 21 and housed either individually (ISO) or in same-sex groups (GRP) of 4 for 4 weeks. Rats were then either exposed to a novel juvenile same-sex rat for 15 minutes or they remained in their home cages. Brains were removed 100 minutes later and underwent immunohistochemistry for c-fos. Counts of c-fos positive cells were performed in the posterior cingulate, piriform and insular cortices; striatum, nucleus accumbens, lateral septum, and lateral habenula. Social experience produced a sex- and region-dependent increase in c-fos in GRP rats that was blunted in ISO rats. Interestingly, the effect of social experience on c-fos was much more pronounced in male than in female GRP rats. In GRP males, the increases were greatest in cortical regions, the lateral septum, and the habenula while in GRP females, significant increases were only observed in the habenula. In ISO males and females, 15 min social experience had no effect on c-fos in any brain region. Taken together, these results suggest that the attenuated activation previously observed after social experience in isolation-reared rats is not specific to the mPFC, but reflects a similar pattern throughout several emotion-related brain regions. Furthermore, they suggest that social experience differentially activates the forebrains of male and females.

2. Voluntary exercise during fear extinction reduces fear renewal: Role for activation of reward circuitry during extinction.
   COURTNEY A BOUCHET¹, A Mika², KG Spence², JE Hellwinkel², S Campeau³, HEW Day³, M Fleshner², BN Greenwood². ¹Univ. of Colorado, Boulder, CO; ²Integrative Physiol., ³Psychology and Neuroscience.

Recent efforts to treat anxiety and fear disorders focus on fear extinction. Exposure therapy in humans has limited efficacy, however, because fear memories often resurface when the extinguished conditioned stimulus (CS) is presented in a new context (fear renewal). Emerging evidence suggests that extinction involves both cognitive and affective processes. Cognitive extinction processes could support learning of a new CS-NoUS association; whereas affective processes could support the learning of a new (less aversive) emotional state assigned to the CS. Manipulations that target both the cognitive (such as noradrenergic (NE) signaling) and affective (such as activation of striatal reward circuits) extinction processes could strengthen extinction memories and make them resistant to contextual modulation during renewal. Acute exercise is both arousing (elevates NE) and rewarding (activates striatal reward circuits); thus could potentially act as an adjunct therapy during extinction to reduce fear renewal. To address this possibility, rats without or with 3 nights of experience with running wheels, were conditioned to fear a tone CS. Fear extinction took place the next day in the familiar, locked or mobile running wheels. Run rats ran during extinction, and this running was associated with a reduction in renewal, as tested in a novel context 1 week later. Administration of naloxone (to block exercise reward) immediately prior to extinction reduced the effect of running during extinction on renewal. These data suggest that exercise during extinction, but not a history of exercise per se, reduces renewal of extinguished fear through a mechanism involving exercise reward. Consistent with this possibility, double fluorescent in situ hybridization revealed that CS exposure during renewal elicited greater activity of the reward-associated direct pathway and an attenuation of activity of the aversion-associated indirect pathway of the striatum in rats that ran during extinction, relative to Locked rats. These data suggest that recruiting an affective extinction process could result in an extinction memory that is less susceptible to contextual modulation.

EXPERTISE: In situ hybridization (radioactive and double Fluorescent In Situ Hybridization), behavior testing

WAYNE BRINER.

From the Department of Psychology, Ashford University, Aurora, NE.

Statistical analysis of experimental work is essential to objectively evaluate outcomes. However, the correct use and interpretation of statistics is important to avoid misleading results. To determine how often statistical errors are seen in neuroscience papers we surveyed 30 recently published papers for the appropriate use of statistical errors. Of the surveyed papers only 4 did not demonstrate an error in the design or execution of their statistical analysis. Nearly all statistical errors fell into three categories, low power, inflated chance of type I error, and inappropriate use of the SEM. These types of errors produce a sense of overconfidence in the findings and may be the source of an apparent lack of reliability in many studies. Solutions include increasing sample sizes, improved statistics education, and demand from manuscript reviewers for the appropriate use of statistics.

EXPERTISE: behavior, light microscopy, pharmacology, statistics

4. High-Fat Diet Attenuates Motivated Behavior

PAIGE M. DINGESS, BJ Anderson, RA Darling, TE Brown.

From the Department of Neuroscience, School of Pharmacy, University of Wyoming, Laramie, WY.

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 high-fat 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, which measures 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 were no significant differences between diet groups and any activity-induced condition. However at 3 weeks, there was a significant decrease in locomotor activity in the HF ad libitum 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 (NC: 30,342 ± 3241, n=9; HF restricted: 20,286 ± 2,623, n=14). 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 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 instrumental responding (429 ± 50) compared to the NC controls (858 ± 45). Overall, our results lead us to hypothesize that exposure to the HF diet elicits a hypo-responsiveness of reward circuitry, which influences appetitive seeking behavior.

EXPERTISE: behavior testing (Conditioned Place Preference, Self-Administration, Locomotor Boxes)

5. Mate preference in female bengalese finches: behavioral and neural influences.

JEFF L DUNNING¹, S Pant¹, K Murphy¹, AA Krentzel², L Remage-Healey², A Bass¹, Z Coburn¹, JF Prather¹.

From the ¹Dept. Zoology and Physiology, Univ. of Wyoming, Laramie, WY; ²Dept. Psychology, Univ. of Massachusetts Amherst, Amherst, MA.

Mate choice is the cognitive event of sexual selection, yet it remains unknown how that decision is encoded in the brain. In many species, such as the songbirds studied here, signals driving mate choice consist of elaborate male displays that are evaluated by females and used as the basis for selecting one mate from among many suitors. The importance of such signals is especially clear in the case of song, as female songbirds will solicit copulation in response to songs played through a speaker, even if no male is present. Previous studies of male song have revealed many features that are modulated in the presence or absence of a female receiver, revealing candidate parameters that may influence female preference. In addition, studies of female response to male song have implicated specific brain sites, such as the caudal mesopallium (CM), in the expression of female mate preferences. We tested the mate preferences of adult female Bengalese finches (Lonchura striata domestica) using songs recorded from 8 different conspecific adult males. Female preferences for specific songs are evident in both copulation solicitation displays and calls in response to song stimuli, and individual preferences are consistent across time and tests. Females vary in their preference for specific males, but comparison across females reveals that specific males are more preferred than others, and
those preferences are evident regardless of whether the females have ever had social experience with the associated singers. Analysis of those broadly preferred songs and additional tests using manipulation of each female’s most-preferred song reveal roles for song tempo and complexity of song sequence in directing female preference. Consistent with previous results from other species, initial findings indicate that levels of immediate early gene expression are elevated in the caudal mesopallium (CM) when the female hears conspecific song. Recordings of CM neurons in freely behaving females engaged in song perception indicate that those cells are more active in response to the female’s most-preferred song than to other songs.

**EXPERTISE:** Behavior Testing, brain microinjections

6. Acute and repeated social defeat differentially activate hypothalamic orexin/hypocretin and melanin-concentrating hormone neurons in the male rat.

ED Paul, BRANDON KK FIELDS, JD Heinze, MW Hale, JL Lukkes, IA Kerman, CA Lowry.
From the University of Colorado Boulder, Department of Integrative Physiology and Center for Neuroscience, Boulder, CO.
The hypothalamic neuropeptides orexin/hypocretin and melanin-concentrating hormone (MCH) are implicated in mediating proactive and passive-submissive (i.e. reactive) coping strategies during stress, respectively. We previously reported that exposure to repeated social defeat stress results in a shift from a proactive coping style to a more reactive coping style that was associated with altered serotonergic activity. In order to determine the effects of social defeat stress on orexin and MCH neuronal activity, we used tissue harvested from Long-Evans male rats that were previously exposed to either acute social defeat (AD), repeated social defeat (RD; i.e., two defeat sessions separated by 24 hr) or home cage control conditions (HCC) to immunohistochemically stain for the neuronal activity marker, c-Fos, in orexin- or MCH-immunoreactive neurons. Both AD and RD, relative to HCC, increased c-Fos expression in orexin neurons, and this effect was significantly greater in AD rats, compared to RD rats. In regards to MCH, exposure to RD and AD, relative to HCC, elevated c-Fos expression in MCH neurons, with a more pronounced effect in RD animals. Neither AD nor RD treatments altered the total number of orexin- or MCH-immunoreactive neurons. Activation of either orexin or MCH neurons was not correlated with any specific coping strategy, but their neuronal activation was associated with alterations in individual behaviors. Elevated orexin activity was positively correlated with rearing behavior, an exploratory escape behavior associated with proactive coping, and locomotion, indicative of behavioral arousal. Increased MCH activity was positively correlated with escape, a proactive behavior, and negatively correlated with self-grooming, a neutral behavior. Finally, activation of orexin and MCH neurons was correlated with neuronal activity in subpopulations of serotonergic neurons of the dorsal raphe nucleus. These data suggest that AD and RD differentially activate orexin and MCH neurons, and this activation may be relevant for changes in behavioral coping strategies possibly via interactions with midbrain serotonergic systems.

**EXPERTISE:** tissue sectioning/mounting, HPLC-ED analysis, light microscopy

7. The rewarding effects of exercise do not depend on wheel running controllability

JONATHAN J. HERRERA, PJ Clark, PR Ghasem, SM Engel, TA Wieman, BN Greenwood, M Fleshner.
From Dept. of Integrative Physiology and Center for Neuroscience, University of Colorado, Boulder, CO.
The mesolimbic reward pathway is implicated in the development and treatment of stress-related psychiatric disorders. Exercise can reduce the incidence of stress-related disorders, but the contribution of exercise reward to exercise-induced stress resistance is unknown. We’ve reported the anxiolytic and antidepressant-like effects of exercise are independent of exercise controllability; whereby both voluntary and forced wheel running (WR) protect rats against behavioral consequences of stress. Voluntary exercise is a natural reward, but whether rats find forced WR rewarding is unknown. The goal of the current studies was to determine whether the rewarding effects of WR depend on its controllability. Following 1 wk of voluntary running, mF344 rats were divided into voluntary and forced groups. For 5 wks, rats were placed into their assigned voluntary or forced wheels or, on alternating nights, into alternate home cages. Two hrs after wheel or alternate home cage exposure, rats were placed on a distinct side of a conditioned place preference (CPP) chamber. One side was paired with running (paired) and the opposite side with the alternative home cage (unpaired). Probe tests to determine CPP were conducted 1, 3, and 5 wks after the start of CPP training. After the final probe trial, and 24 hrs after the last running bout, rats were placed on either the paired or unpaired side and sacrificed 30 min later. Double in situ hybridization (FISH) was used to assess potential conditioned activation of reward circuitry elicited by acute exposure to the paired side. Rats spent more time on the side of the CPP chamber paired with exercise during each probe trial, regardless of WR controllability. Furthermore, FISH analyses of rats
paired with running showed greater activation of direct pathway dynorphin neurons (a potential signal
mediating reward), while rats paired with a sedentary context demonstrated greater activation of indirect
pathway enkephalin neurons (a potential signal mediating aversion). Together, these data suggest that both
voluntary and forced WR can be rewarding. The rewarding effects of exercise could contribute to the
mechanisms by which exercise increases stress resistance.

**EXPERTISE:** Conditioned Placed Preference (CPP) & fluorescence in-situ hybridization (FISH)

8. Error Monitoring and Working Memory Load: Presence and Alteration of a Late Positivity in
Stimulus-Locked ERPs

**BENJAMIN T JOHNSON**, **BK Taylor**, **PL Davies**, **WJ Gavin**.

From the 1Molecular, Cellular, and Integrative Neurosciences, Colorado State University, Fort Collins, CO;
2Human Development and Family Studies, Colorado State University, Fort Collins, CO; 3Department of
Occupational Therapy, Colorado State University, Fort Collins, CO.

Error-related negativity (ERN; also referred to as the Ne) is an event related potential (ERP) recorded by
electroencephalogram (EEG) that is characterized as a response-locked negative deflection in voltage
following an incorrect response to a given task. In this study we utilized three different paradigms that varied in
working memory (WM) load to test whether or not WM affected ERN amplitude. We hypothesized that the ERN
would not be impacted by WM load, consistent with the hypothesis that the ERN is a generic performance
monitoring signal. Here we show that WM load does not affect baseline-to-peak measured ERN amplitudes but
does attenuate the post-ERN positivity (PE) $F(2, 48) = 11.99, p < 0.005, \eta^2_p = 0.33$. In addition, observations
within stimulus-locked recordings revealed a significantly greater negative N2 (a stimulus-locked ERP
component associated with attention) amplitude for correct trials compared to incorrect trials $F(1, 27) = 87.41,\
p < 0.005, \eta^2_p = 0.76$. N2 latency was faster for incorrect trails for the zero and low WM load task but no
difference was present in latency between correct and incorrect trails for the high WM task. A late positivity
was also present as a function of the two-rule, high WM load paradigm utilized and this additional brain
processing was diminished in the case of errors $F(1, 27) = 21.15, p < 0.005, \eta^2_p = 0.44$. As WM load did not
impact the ERN amplitude, these findings support the hypothesis that the ERN exists as a generic
performance monitoring signal. However, WM load and the occurrence of errors did impact ERP components
reflecting attention and evaluation of responses, and altered later brain processing.

9. Effects of menstrual cycle phase and oral contraceptive use on perception of emotional stimuli with
reproductive significance: An event-related potential study.

**POLINA A. REYNOLDS**, **MA Kisley**.

From the Psychology Department, University of Colorado, Colorado Springs, CO.

Perceptual differences, particularly those related to courtship and mating, have been discovered across the
menstrual cycle. Oral contraceptives (OCs) are used by a significant portion of childbearing age women to alter
their cycles. This study assessed event related potentials (ERPs) and evaluative categorization responses to,
and subjective ratings of images with and without reproductive significance in women who were naturally
cycling and those on monophasic formulations of hormonal birth control. Both groups were tested during days
13-20 (ovulation and luteal) of their cycle and previous 3 months of menstrual cycle data was used to estimate
day of ovulation for study cycle and control for progesterone level. The IAPS database was used for all images;
neutral people served as context images and three categories of images with positive valence were chosen as
targets (men, babies, and romance). Images were presented to participants to categorize as not positive,
somewhat positive, or very positive with targets presented pseudo-randomly among neutral people images.
Quantitative SAM ratings of valence and arousal were obtained for each of the target images at the conclusion
of the ERP measurements. P300 amplitudes were compared across groups and no effect of OC use on
processing of reproductive stimuli was found. There was a trend toward reduced ERP amplitude in response to
images of men with OC use, and this should be further investigated with a larger sample size and more
rigorous control of menstrual cycle day.

10. Guppies show significant levels of repeatability in many but not all exhibited behaviors.

**SEAN P STREICH**, **E Fischer**, **K Hoke**.

From the Department of Biology, Colorado State University, Fort Collins, CO.

Repeatability is the measure of variance within individuals that derives total variance of a population. Behavioral repeatability can be extremely variable because it is a phenotype that is affected by many factors including genetics, past experience, and environmental stimulation. The combination of these factors often
contributes to different behaviors being exhibited in response to the same stimulus, both within and among individuals. Evolution and environmental pressures will also act on behavioral mechanisms, resulting in variable expression in some behaviors while leaving others unaffected. Determining whether behaviors are repeatable will allow insight into how regulated behavioral mechanisms are as well as provide validation on the strength data collected from behavioral observations. To investigate behavioral repeatability I developed four assays to measure behavioral situations in guppies (Poecilia reticulate): shoaling, courting, aggression, and escape. All assays were conducted three times per individual over a nine week period. For each situation a suit of behaviors were observed and qualifying data was recorded for each assay. The strength of repeatability was determined for each observed behavior, both for individuals and across all individuals. Though levels varied, I found most behaviors showed significant levels of repeatability regardless of the time between trials. The results show that many behaviors exhibited by our population of guppies are consistently preformed while a few behaviors show great variability in expression.

**EXPERTISE:** behavior testing, live animal handling, cell cultures, plasmid collection, small mammal trapping, drawing blood.

11. Test-retest Reliability of the Visually-Evoked CNV.

BRITTANY K TAYLOR¹, PL Davies², WJ Gavin¹.

From the departments of ¹Human Development & Family Studies, and ²Occupational Therapy, Colorado State University, Fort Collins, CO.

The contingent negative variation (CNV) is an event-related potential derived from electroencephalography (EEG) and is representative of sustained attention in the brain. Specifically, the CNV is a slow, negative drift elicited during Go trials of a Go-NoGo task. A conditional stimulus informs the participant whether a response will be required (Go or NoGo), then after a fixed interstimulus interval (ISI; e.g., 2 seconds), an imperative stimulus cues the participant to respond for Go trials. In the case of NoGo trials, brain activity returns to baseline levels after the initial processing of the conditional stimulus. The CNV is often measured as three components: the O-wave (i.e., orientation), the E-wave (i.e., expectancy), and the total CNV. Very few researchers have examined the test-retest reliability of the CNV and its components, and only in the context of an auditory task. This study assessed the test-retest reliability of the CNV and its components during a visual task. Thirty-two adults performed a simple visual Go-NoGo task while EEG was recorded during two different sessions set one week apart. First, the averaged amplitudes of the three CNV components were measured and compared between Go and NoGo trials for both sessions. The findings indicated that in session 1, adults had significantly larger negative mean averaged amplitudes for the Go trials for the O-wave, t(32) = -2.29, p = .03, the E-wave, t(32) = -7.13, p < .005, and the total CNV, t(32) = -5.58, p < .005. In session 2, only the E-wave, t(32) = -5.33, p < .005, and the total CNV, t(32) = -3.39, p = .002, presented with a larger negative mean amplitudes in Go trials compared to NoGo trials. Results for the test-retest reliability of the CNV components indicated that the O-wave was the most reliable component, r(32) = .58, p = .001, then the E-wave, r(32) = .19, p = .30, and finally the total CNV, r(32) = .05, p = .79. Although adults did not produce an O-wave in Go trails that was significantly different from NoGo trials in session 2, the O-wave still presented with the highest reliability of all the examined components suggesting that the O-wave changed from session 1 to session 2 in a systematic way, which may be indicative of learning.

12. Exercise and stress resistance: Protection of diurnal rhythms and sleep architecture

ROBERT S THOMPSON, BN Greenwood, M Fleshner.

From the University of Colorado at Boulder, Department of Integrative Physiology, and the Center for Neuroscience.

Exercise can increase resistance to stress-related psychiatric disorders such as anxiety and depression. One way exercise may confer stress resistance is by reducing the impact of stress on sleep and diurnal physiological rhythms; disruptions of which are thought to contribute to stress-related disorders. Indeed, exercise is a powerful non-photic entrainment cue to the biological clock and thus could help prevent or reverse stress-induced disruptions in diurnal rhythms. Adult male F344 rats (8 per group) remained sedentary or had voluntary access to wheels for 6 weeks. After 4 weeks of exercise, rats were implanted with F40-EET biotelemetry devices (DSI) and real-time continuous diurnal/circadian rhythms were recorded. Following a 1-week recovery period, rats were again allowed access to running wheels for an additional 2 weeks. Diurnal rhythms of locomotor activity, heart rate, body temperature, and sleep (i.e. REM, NREM, and WAKE) were continually recorded in the presence of a 12hr light/dark cycle in running and sedentary rats. After a total of 6 weeks of exercise, both the sedentary and running rats were exposed to a single acute stressor (100, 5-s, 60-s ITI inescapable tail shock) previously reported to disrupt physiological diurnal rhythms and produce behaviors
resembling symptoms of affective dysregulation. Physiological parameters were measured prior to, during, and following stressor exposure. Compared to sedentary rats, exercise rats had larger diurnal amplitudes of locomotor activity rhythms, core body temperatures and %REM during the light (inactive) cycle prior to stressor exposure. The increase in REM persisted following stress in the exercise rats. During stress, both groups reached maximal heart rate, and physically active rats reached a higher maximum body temperature. Stressor exposure impacted diurnal rhythms of activity, heart rate, body temperature, and sleep in both sedentary and exercise rats; and these changes persisted for 48-96 hours. These data suggest that protection against stress-induced disruptions in diurnal rhythms and increases in REM sleep could contribute to the cognitive and affective benefits of exercise. Funding provided by: NIH RO1 MH068283, DARPA W911NF-10-1-0050

EXPERTISE: telemetry surgery

13. Event-related potentials for implicit, explicit, and empathic processing of emotional facial expressions.

TIEN T TONG, TJ Groth, JS Nomi, S Bastidas, LJ Troup.
From the Psychology Department, Colorado State University, Fort Collins, CO.
The current study examined how implicit, explicit, and empathic responses to emotional facial expressions influence event-related potentials (ERPs). Non-depressed and non-anxious human participants viewed happy, sad, and neutral emotional facial expressions while attempting to either identify the gender (implicit), identify the emotional expression (explicit), or empathize with the emotional expression (empathic). EEG data were recorded from 19 electrodes set according to the international 10-20 system with analysis focusing on mean amplitudes for midline and bilateral frontal, central, and parietal electrodes. Midline analysis showed that the vertex positive potential (VPP; 140-200ms) amplitude was largest for sad faces across all electrodes regardless of condition. Global analysis showed that P3 (200-400ms) amplitudes in the expression recognition condition differed by hemisphere and electrode for sad faces but not happy or neutral faces. Finally, the global analysis also showed that P3 amplitudes for all three emotional expressions in the empathize condition differed by hemisphere and electrode. The results suggest that the early perception of emotional expression across all manipulations is represented by increased midline amplitude of the VPP while processes such as emotional recognition and empathy are represented by differences in P3 amplitudes across hemispheres and electrodes.

EXPERTISE: Behavior testing, EEG/ERP

Development

14. Conditional ablation or raptor or rictor in oligodendrocytes results in differential dysmyelination in the CNS.

From the Department of Cell and Developmental Biology, University of Colorado, Aurora, CO.
Throughout central nervous system (CNS) development, oligodendrocytes undergo multiple transitory stages before terminating in mature, myelinating cells. Many different factors regulate this process, but our lab is interested in understanding the role of protein kinases. Previously, our lab has showed that constitutive activation of Akt in myelinating cells, results in a hypermyelinating phenotype. Through further investigation, it was shown that mTOR was a critical mediator of this phenotype as well as in wildtype mice. The mammalian Target of Rapamycin (mTOR), is a serine threonine protein kinase which exists in two distinct complexes. These complexes are defined as mTOR-Raptor (mTOR complex 1) or mTOR-Rictor (mTORC2). To dissect the role of the individual complexes in the process of oligodendrocyte differentiation and myelination, we are utilizing a myelinating glial cell promoter crossed to either the Raptor (mTORC1) or Rictor (mTORC2) floxed mice. Our data shows that Raptor conditional KnockOut (cKO) mice have a more dramatic loss of myelin throughout development, whereas the Ricto cKO mice do not have a significant myelination defect throughout development. We are further investigating the signaling change in these animals that are a consequence of ablation of either Raptor or Rictor, and how their loss may influence transcriptional changes in oligodendrocytes regulating their distinct phenotypes.

EXPERTISE: Tissue culture, Immunohistochemistry, Immunocytochemistry, co-Immunoprecipitation, Western Blots, real time qPCR, Confocal microscopy, Electron microscopy
15. A developmentally compromised serotonergic system is associated with reduced serotonin neuronal activation in response to stress: the role of fibroblast growth factor 8.
LEAH R BROOKS, RA Woolaver, CL Enix, CA Lowry, PS Tsai.
From the Department of Integrative Physiology, CU Boulder.

Mental disorders such as anxiety and major depression can be exacerbated by stress and are the leading causes of disability from adolescence to adulthood. The prevalence of these disorders necessitates an understanding of how stress- and anxiety-related neurocircuits are regulated throughout an individual's life. Functionally heterogeneous populations of serotonergic neurons, located within the dorsal raphe nucleus (DR), play a role in stress-related behaviors and neuropsychiatric illnesses such as anxiety and depression. Abnormal development of these neurons can permanently alter their structure and connections, making the organism more susceptible to disorders like anxiety. A factor that critically regulates the development of serotonergic neurons is fibroblast growth factor 8 (Fgf8). In mice, a total loss of Fgf8 leads to obliteration of the developing midbrain/hindbrain region and leads to prenatal mortality. However, mice genetically altered to produce 30% less Fgf8 (Fgf8 hypomorphs) survive normally. We have previously shown that Fgf8 hypomorphs exhibit a blunted behavioral response to stress. In order to elucidate whether this behavioral response is associated with functional changes in the serotonergic system, brains were collected from wildtype mice and Fgf8 hypomorphs that had been exposed to one hour of restraint stress or no stress. The brains were immediately fixed in paraformaldehyde and processed for immunohistochemistry. Five 30-µm sections spanning the DR were used to quantify the percent of cells co-expressing c-Fos (an immediate-early gene) and tryptophan hydroxylase (a marker of serotonergic neurons) in the ventral (DRV) and ventrolateral wing (DRVL) subregions of the DR. Our results revealed a significant main effect of stress in the midrostrocaudal DRV and DRVL and genotype x stress interactions in the DRVL and DRV. The DRVL neuronal activation results mirror the blunted behavioral stress response in Fgf8 hypomorphs. Overall, our results provide a neural substrate upon which Fgf8 deficiency could affect stress response and support the hypothesis that developmental disruptions of serotonergic neurons affect their functional integrity.

16. Identification of the gene implicated in the zebrafish macho mutant
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The structural subunits of voltage-gated sodium channels have been identified, however less is known about mechanisms that regulate their function in vivo. In the zebrafish touch-insensitive mutant macho (mao), sensory neurons have reduced sodium current amplitudes. In previous work, we found that the mao mutation is not linked with sodium channel α-subunit or β-subunit genes, indicating that the lesioned gene does not code for a structural subunit. Here, we report that mao mutants carry a genetic lesion within the pigk gene, consisting of a T to C substitution in the start codon. The pigk gene codes for the protein Pigk, an transposase critical to attaching glycosylphosphatidylinositol (GPI) anchored proteins (GPI-AP) to their GPI-anchor and allowing the GPI-AP to be tethered to the extracellular cell surface. Over expression of wildtype pigk mRNA in mao mutants resulted in mao mutants with a touch response, indicating the pigk mutation underlies the mao phenotype. This result was surprising as sodium channel subunits are not GPI-anchored. Using qPCR, we assessed pigk mRNA levels during embryogenesis and found that it is both maternally and zygotically expressed, and present throughout the first 48 hours post fertilization. Using RNA in situ hybridization we characterized the spatial expression pattern of pigk during early development and found that the gene is ubiquitously expressed in the dorsal spinal cord, where the sensory neurons reside. The implication of this gene in the mao mutant identifies a potentially novel mechanism for regulation of sodium current (INa) amplitude. Further studies can distinguish the mechanisms in which this gene specifically attenuates the INa amplitude in order to better understand the physiological relevance of this mutation.

EXPERTISE: immunocytochemistry, in situ hybridization
Pericyte morphology differs by brain region in mice: Focus on the paraventricular nucleus of the hypothalamus

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The paraventricular nucleus of the hypothalamus (PVN) is a dense collection of neurons that play key roles in maintaining homeostasis and initiating stress responses. It is also characterized by a dense matrix of blood vessels compared to surrounding brain regions. In mice, the additional vasculature arises between postnatal days (P)12 and P20. To examine whether the postnatal angiogenic period impacted blood-brain barrier (BBB) competency, extravascular fluorescein isothiocyanate (FITC) leakage and pericytes as key cellular elements of neurovascular units were examined. Specifically, pericyte morphology and distribution within the PVN were compared to the parietal Cortex (CTX). On P12, 22, and 52 FVB/N mice were perfused transcardially with heparin PBS containing FITC followed by 4% paraformaldehyde to visualize functional blood vessels. Immunoreactive desmin, an intermediate filament protein in pericytes, was used to visualize morphology. Confocal images of the PVN and CTX were collected to view extravascular FITC leakage and pericyte distribution in relation blood vessels delineated by FITC. Results showed a significant level of extravascular FITC, indicating leakage specifically within the CTX at P12 compared to the PVN (p < 0.05). At P20, there was no significant extravascular FITC leakage within the PVN indicating the postnatal angiogenic period did not impact BBB competency. There was significantly more immunoreactive desmin in the PVN compared to the CTX (p < 0.01) at all ages examined. When blood vessel density was taken into account, there was still significantly more immunoreactive desmin in the PVN compared to the CTX (p < 0.01). For the PVN, there was also a significantly more immunoreactive desmin at P22 compared to P12 (p < 0.01) that was maintained into adulthood (P52). Blood vessels in the PVN had a significantly greater average width compared to the CTX (p < 0.05). The pericytes, based on immunoreactive desmin, appeared to elongate only down blood vessels in the CTX and a small subset of blood vessels within the PVN. The larger number of pericytes within the PVN showed a wrapping pattern on larger vessels. Overall, blood vessels and BBB competency and components varied among different brain regions. Further examination may provide insight into BBB development and whether certain conditions and diseases impact particular brain regions with a different incidence at specific times.

EXPERTISE: immunohistochemistry, behavior testing, blood-brain barrier investigation

The persistence of exercise-induced stress resistance depends on the developmental stage during which exercise is initiated.

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Exercise reduces the incidence of stress-related psychiatric disorders in humans and prevents the development of anxiety- and depressive-like behaviors in rodents, including exaggerated fear and deficits in shuttle box escape learning. When wheel running is initiated around puberty (PND 45-49), the protective effects of exercise persist for 15 days, but are lost by 25 days following forced cessation of exercise. Many studies show the brain is extremely plastic in early life, such that positive and negative manipulations during this sensitive period produce long lasting changes in brain plasticity and behavior. The stress-protective effects of exercise during sensitive periods of development are unknown. We therefore examined the effects of early life exercise on the persistence of the stress-protective effects of voluntary wheel running following exercise cessation. Adult (PND 70) and juvenile (PND 24) male, Fisher (F344) rats where allowed access to a running wheel or remained sedentary for 6 weeks. All wheels were then rendered immobile, and rats were exposed to no stress or uncontrollable stress 14 or 24 days later. 24 h following uncontrollable stress, behavioral testing for shock-elicited fear and shuttle box escape occurred, so that exercised rats were forced to remain sedentary for either 15 or 25 days prior to testing. When wheel running was initiated during adulthood (PND 70), the protective effects of exercise on exaggerated fear and shuttle-box escape deficits were lost by 15 days. Interestingly, when wheel running was initiated during the juvenile period (PND 24), the protective effects of exercise persisted longer, and showed no signs of attenuating at 25 days. These results suggest that exercise during early sensitive developmental periods can alter the trajectory of brain development to produce long-lasting stress resistance to the behavioral consequences of exposure to traumatic events during adulthood. EXPERTISE: In situ hybridization, behavior testing
19. Impacts on input and output neurons of the suprachiasmatic nuclei in fibroblast growth factor 8 deficient mice.

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Fibroblast growth factor (FGF) 8 is 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 deficiency also impacts the integrity of the suprachiasmatic nuclei (SCN). The SCN is a principal regulator of the organism’s circadian rhythm and consists of neurons that produce vasoactive intestinal peptide (VIP) and vasopressin (AVP) as main input and output neurons, respectively. The objective of this study is to examine the number of VIP and AVP neurons in the SCN of postnatal day (PN) 0 and PN 60 mice hypomorphic for Fgf8. Brains from wildtype mice (WT), heterozygous Fgf8 hypomorphs (HET), and homozygous Fgf8 hypomorphs (HOMO) were fixed in acrolein (for PN0) or 4% paraformaldehyde (for PN60), sectioned in a cryostat, and processed for VIP and AVP immunohistochemistry. The number of VIP- and AVP-immunoreactive (ir) neurons was quantified in the SCN. On PN0, both VIP and AVP-ir neurons were significantly reduced in the SCN of HET and HOMO mice, with the greatest reduction occurring in HOMO mice. Since HOMO mice do not survive past PN0, only WT and HET mice were compared on PN60. The reduction in AVP-ir neurons in the SCN persisted in PN60 HET mice, but VIP-ir neurons were no longer significantly reduced in these mice. These results show that Fgf8 deficiency leads to a permanent and irreversible loss of SCN AVP-ir neurons but only transiently delays the maturation of SCN VIP-ir neurons. Importantly, these data suggest Fgf8 deficiency can impact the structural integrity of the SCN via multiple mechanisms.

EXPERTISE: immunocytochemistry, PCR

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

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It is well established that long-term changes in synaptic structure and function are mediated by rapid activity-dependent gene transcription and new protein synthesis. A growing body of evidence supports the involvement of the microRNA (miRNA) pathway in these processes. We have used the Drosophila neuromuscular junction (NMJ) as a model synapse to characterize activity-regulated miRNAs and their important mRNA targets. Here, we have identified five neuronal miRNAs (miRs-1, -8, -289, -314, and -958) that are significantly downregulated in response to neuronal activity. Furthermore we have discovered that neuronal misexpression of three of these miRNAs (miR-8, -289, and -958) is capable of suppressing new synaptic growth in response to activity suggesting that these miRNAs control the translation of biologically relevant target mRNAs. Interestingly, one predicted target is the Ca2+/calmodulin-dependent protein kinase II (CamKII). CamKII is known to respond in an activity-dependent manner in the Drosophila olfactory system and is regulated by the miRNA pathway in this system (Ashraf et al., 2006). While CamKII has a well-characterized postsynaptic function, its presynaptic role is less understood. We find that the CamKII 3'UTR is regulated by miR-289 in-vitro and this regulation is alleviated by mutating the 'seed region' of the miR-289 binding site within the CamKII 3'UTR. Using an antibody for CamKII we see presynaptic staining in a punctate pattern with strong colocalization with active zone markers. Furthermore, we observe a significant increase in mean CamKII fluorescence levels in response to spaced synaptic depolarization indicating a increased expression of CamKII in response to activity. Finally, we have investigated the presynaptic overexpression of several CamKII isoforms to examine the effects of calcium dependence on new synaptic growth in response to spaced synaptic depolarization.


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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 sensory epithelium undergo neurogenesis, integration, and turnover even into adulthood, making it an ideal model to study neuronal development. Here we determine the effect of PrPC level on neurodevelopment under three conditions: 1) homeostasis, 2) acute injury/synchronized regeneration, and 3) prion-induced neurodegeneration. To investigate the role of PrPC in OSN proliferation, dividing cells were quantified using BrdU incorporation in adult wildtype, PrP-overexpressing, and PrP-null mice. Results indicate that PrPC plays a role in maintaining mature OSNs within the epithelium, whereby an absence of endogenous prion resulted in an increased cell number at one week, but over time fewer of these dividing cells were maintained in the OSE. Next, using quantitative real-time PCR (qPCR), we quantified gene expression indicative of OSN differentiation. In mice overexpressing PrPC, there was an increase in expression of mature neuronal markers, which may be due to decreased neuronal turnover. Loss of PrPC had only a modest effect on differentiation, which suggests a redundant role for OSN specification within the OSE. Together, these data indicate a putative neuroprotective role for PrPC in adult OSE neurogenesis, whereby more mature neurons are stably maintained in animals expressing PrPC.

Disorders of the Nervous System

22. A potential compensatory role for endogenous striatal tyrosine hydroxylase-positive neurons in a non-human primate model of Parkinson's disease.

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The neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) selectively destroys dopaminergic (DA) neurons in the nigrostriatal pathway while importantly sparing other DA systems, serving as a significant tool in Parkinson’s disease (PD) research. Extensive studies in our laboratories have shown reversal of MPTP-induced PD symptoms following striatal grafts of fetal ventral mesencephalic DA neurons in the African Green monkey. Additionally, our and other studies have reported an upregulation of endogenous tyrosine hydroxylase (TH) positive neurons in the striatum following MPTP lesions (~140% increase). The aim of the current research is to investigate the fate of this endogenous population in MPTP-treated monkeys following fetal cell grafts as well as their relationship to symptom severity. Preliminary results indicate a return to control levels for the TH-positive cells following successful transplantation, while animals whose donor cell grafts failed to contain DA neurons retained the elevated levels. Additionally, we found the increase in cell number remained constant between animals with mild, moderate and severe symptoms, while asymptomatic subjects retained control levels. If these cells represent a compensatory mechanism in an attempt to replenish DA in the striatum, then future studies aiming to influence this population could prove beneficial in developing new non-invasive therapeutic treatments. Supported by an Academic Enrichment Grant from the University of Colorado School of Medicine, The Axion Research Foundation, and 5PO1NS044281.

23. Inhibition of hypothalamic POMC neurons increases food intake in an activity-based anorexia mouse model.

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Anorexia is a serious condition that has deleterious health effects and increases an individual’s risk of mortality. A common feature seen with anorexia, particularly anorexia nervosa (AN), is an increase in physical activity despite the severely reduced caloric intake. Physiological mechanisms that contribute to anorexia and increased physical activity are not fully understood. Peptides released from proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus decrease food intake, activate reward pathways, and increase energy expenditure. POMC neurons have also been shown to have a role during activity-based anorexia (ABA) and AN. Previous research and preliminary data show a transient increase in POMc mRNA during the onset of ABA in rats and mice respectively. However, it is not known if this increase in POMc message is a necessary step in the development of ABA. The purpose of this study is to determine if silencing POMC neurons delays the progression of ABA. The inhibitory DREADD (hM4Di [Designer Receptor Exclusively Activated by Designer Drug]) receptor was used to decrease POMC neuron activity during ABA. The ability of this receptor to selectively target to POMC neurons and selectively decrease POMC neuron activity was shown using immunohistochemistry and electrophysiology. Mice develop ABA over the course of 3
days when 2-hr food access (FA) is paired with voluntary wheel running. These animals lose significantly more weight than food restricted sedentary mice. Also, wheel-running activity significantly increases during ABA. To determine if inhibition of POMC neurons increases food intake during ABA, mice expressing hM4Di received 2 daily injections (i.p.) of clozapine-n-oxide (CNO), the ligand for hM4Di, during the development of ABA. When POMC neurons were inhibited in this way, mice consumed significantly more food on day 3 of ABA relative to control mice not expressing hM4Di. These data verify that mice develop the key behavioral features of ABA and suggest that mice have a transient increase in Pomc mRNA during ABA similar to that seen in rats. CNO-mediated inhibition of POMC neurons decreases cell activity in vitro and increases food intake during ABA. These results further support the role of hypothalamic POMC neurons in the development of ABA.

**EXPERTISE:** Stereotaxic injection, immunohistochemistry, behavior testing, electrophysiology

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**24. Brain metabolomics of rapamycin treatment in the Ts65Dn mouse model of premature aging and Down syndrome.**

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Aging is often associated with impaired cognition and a progressive loss of organ function over time accompanied by an increased susceptibility for many disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), heart disease, osteoporosis, type II diabetes, and many forms of cancer. Interventions that can increase the health span of an individual are of the utmost importance. With a rapidly aging population, the negative impacts of aging and age-related disorders is a major cause of increased human suffering both for affected individuals and for families and caregivers. Metabolic changes are also apparent in normal aging, but may increase in magnitude or nature with accompanying disease states or with accelerated aging. Thus, studying aging in a diseased state, or in a disorder characterized by accelerated aging, will facilitate identification of these changes. Down syndrome (DS) is an intellectual disability characterized by premature aging. We hypothesize that trisomy of chromosome 21 (HSA 21) causes disruption of the metabolome leading to an accelerated aging phenotype. In the Ts65Dn mouse model of DS, a premature aging phenotype is also observed. We propose using the Ts65Dn mouse model to study the metabolic changes associated with trisomy, and how these change with age. An initiative by the National Institute on Aging (NIA) Interventions Testing Program testing the efficacy of rapamycin treatment demonstrated that mice treated with rapamycin showed a significant increase in lifespan and health span. Therefore, we hypothesize that treatment of the Ts65Dn mouse model of premature aging and DS with rapamycin will ameliorate the accelerated aging phenotype in the Ts65Dn mouse model of DS.

**EXPERTISE:** HPLC, electrochemical detection metabolomics, qPCR, tissue culture, Western Blot

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**25. MicroRNA expression during differentiation of embryonic stem cells to midbrain dopamine neurons.**

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Parkinson's Disease (PD) is the second most common form of neurodegeneration in elderly populations with the principal impediment being the progressive degeneration of midbrain dopaminergic neurons (mDNs) as a result of oxidative stress, α-synuclein aggregation (Lewy Bodies), genetic predisposition and epigenetic elements. The main course of treatment has been a daily dose of costly medication, however; in recent years transplantation of differentiated embryonic stem cells (ESCs) into the putamen has emerged as a promising alternative to the daily regime of drugs, not only halting the progression of the disease but also reversing several of the clinical symptoms of PD. Current methods of differentiating ESCs to mDNs can take upwards of 20+ days with only 30% efficiency; therefore, a more efficient method of differentiation is needed. In recent years the discovery of highly conserved, small (18- to 25-nucleotide), non-protein coding sequences of RNA, known as microRNA (miRNAs), have been shown to regulate gene expression during development of the mammalian central nervous system. MicroRNAs regulate cell fate decisions by silencing and directing the degradation of mRNA, possibly providing a model for quicker and more efficient differentiation methods in vitro. Using two green fluorescent protein (GFP) cell lines and traditional methods of differentiation, cells in three different stages of differentiation (ESCs, neural stem cells, and mDNs) were isolated via fluorescent activated cell sorting (FACS). MicroRNAs were then extracted and sequenced to elucidate microRNA expression during differentiation of midbrain dopamine neurons from embryonic stem cells.

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Stroke is a sexually dimorphic disease that affects men more severely than women. We recently demonstrated that pharmacological and genetic inhibition of TRPM2, a member of the transient receptor potential (TRP) channel superfamily, protects against cerebral ischemia in male mice, while having no effect in females. TRPM2 channels are calcium permeable non-selective cation channels activated by hydrogen peroxide. Using a novel peptide, tat-M2NX, we show specific inhibition of TRPM2 activation by hydrogen peroxide in vitro. Animals treated with the tat-M2NX peptide 4 hours after onset of middle cerebral artery occlusion (MCAO) had significantly smaller infarcts compared to vehicle treated animals. In addition, TRPM2-/- mice exhibited smaller infarcts compared to wild-type animals, and no further reduction in infarct size was observed in TRPM2-/- mice that were administered the tat-M2NX peptide. These data indicate that inhibition of the TRPM2 channel provides protection from ischemic stroke in male animals, and the tat-M2NX TRPM2 inhibitor may represent a new therapeutic approach with a relatively wide window of opportunity.

EXPERTISE: Tissue culture, immunohistochemistry

27. TAR DNA-binding protein-43 localization and function are coupled to mitochondrial physiology

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Protein aggregation is a common pathogenic mechanism in diverse neurodegenerative disorders. TAR DNA-binding protein-43 (TDP-43) cytoplasmic inclusions are pathological hallmarks of several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal lobe degeneration (FTLD). Furthermore, TDP-43 mutations have been identified in both familial and sporadic forms of ALS. Despite evidence linking dysfuction of TDP-43 to neuronal cell death, it is currently unknown whether TDP-43-induced toxicity occurs via a gain of function or loss of function mechanism. In the present study, we initially examined the effects of either overexpression or knockdown of TDP-43 on neuronal survival in vitro, neither of which induced overt neuronal cell death. However, a significant decrease in the function of mitochondrial ETC Complex I occurred following TDP-43 knockdown in NSC34 motor neuron/neuroblastoma hybrid cells. We also noted that both endogenous and overexpressed TDP-43 partially localized to mitochondria within diverse neuronal and astrocytic cell types. Furthermore, TDP-43 nuclear export was enhanced by reductions in mitochondrial glutathione (GSH) levels. Accordingly, redistribution of TDP-43 from the nucleus to the cytoplasm was induced by ethacrynic acid and this effect was inhibited by overexpression of the mitochondrial GSH transporter, the 2-oxoglutarate carrier, which specifically increased the mitochondrial GSH pool. Our data suggest a novel link between TDP-43 localization and function and mitochondrial physiology. Based on the known role of TDP-43 in regulating mRNA stability and splicing, we hypothesize that mitochondrially-localized TDP-43 may regulate translation of mitochondrially-encoded ETC subunits, specifically Complex I. We propose a model in which decreased mitochondrial GSH levels and increased mitochondrial oxidative stress trigger nuclear export of TDP-43, some of which aggregates in the cytoplasm and some of which localizes to mitochondria. Mitochondrial TDP-43 may provide a compensatory loop to rescue ETC dysfunction by facilitating the translation of mitochondrially-encoded Complex I subunits.

EXPERTISE: Western Blotting, ICC, qPCR, tissue culture, live/dead assays

28. The Nurr1 ligand 1,1-bis(3′-indolyl)-1-(p-chlorophenyl) methane (C-DIM12) supports a dopaminergic phenotype in multiple neuronal cell lines.

SEAN L HAMMOND, S Safe, R Tjalkens.
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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 regulates 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. With many known functions of Nurr1, an endogenous
ligand for activation 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 multiple dopaminergic neuronal cell lines (N2A, N27, MN9D) 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 real time qPCR showed modest induction of Nurr1 expression in several neuronal cell lines but significantly induced Nurr1-regulated genes with C-DIM12 in a time- and dose-dependent manner. C-DIM12 increased expression of Nurr1 in N2A cells overexpressing human Flag-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.

EXPERTISE: real time qPCR, IF staining

29. Exploring the role of the nucleus tractus solitarius proopiomelanocortin neurons in the regulation of energy balance

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Obesity is a national health issue affecting more than two-thirds of the population. Studying the neuronal circuitry underlying this condition is an important step to developing effective treatments. Proopiomelanocortin (POMC) neurons in the central nervous system play a key role in energy regulation. When POMC neurons are ablated or malfunction, the animal becomes obese. POMC neurons have been mapped and phenotyped in the arcuate nucleus of the hypothalamus, where they regulate many aspects of food intake and reward. POMC neurons in the solitary tract nucleus (NTS) of the brainstem are not as well understood due to the difficulty in studying these neurons where POMC peptide expression declines to undetectable levels shortly after birth. Transgenic mice expressing green fluorescent protein (GFP) driven by the POMC promoter can be used to identify POMC cells even after POMC mRNA levels are undetectable. The expression of GFP and electrophysiological studies indicate that POMC neurons are still active in postnatal life, but no longer function to release the typical POMC peptide transmitters α-MSH, β-endorphin, or ACTH. Thus, studies were conducted to determine what amino acid transmitters these neurons may release in adult mice. Fluorescent in-situ hybridization was used to detect the vesicular transporter for glutamate (vGlut-1 and vGlut-2) and the GABA producing enzymes (Gad-65 and Gad-67) in the NTS of POMC-eGFP mice. The results show that NTS cells labeled with POMC-eGFP are heterogeneous and can be either glutamatergic or GABAergic. Reconsidering the function of these neurons in terms of their amino acid transmitter phenotype may provide important insight into their physiologic function and their regulation of energy balance.

30. A protective dose of selenium changes expression levels of select selenoprotein transcripts in N171-82Q Huntington’s disease mouse brain.

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Huntington’s disease (HD) is a progressive neurologic disorder caused by polyglutamine-expanded mutant huntingtin protein (mhtt). Selenium is an essential nutrient that is required for normal brain function. Results from our lab indicate that selenium supplementation protects against declines in motor endurance and brain mass as well as decreases mutant huntingtin aggregate levels. The function of selenium in brain is mainly mediated by selenoproteins. The purpose of this study was to determine whether our selenium intervention increases selenoprotein-encoding transcripts in the brains of N171-82Q HD mice. Female HD and wild-type littermate were treated with selenite in drinking water or received control water from 6-14 weeks of age. Quantitative real-time PCR showed that selenium supplemented N171-82Q HD mice had a significant increase in brain glutathione peroxidase 3 (GPX3) mRNA and decreased selenoprotein V mRNA compared to HD controls. Further, for several genes the effect of selenium supplementation was genotype-dependent. Our findings show differential transcriptional responses to selenium supplementation between wild-type and HD mice and suggest that some selenoprotein-encoding genes could be mediating the protective effects of selenium supplementation in HD mice.

EXPERTISE: real time qPCR

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Currently, there are no approved therapeutics for Parkinson’s disease (PD) that prevent inflammatory injury to neurons. PD presents with high levels of neuroinflammation within the nigro-striatal track leading to a progressive loss of dopamine neurons in the substantia nigra (SN). This study examined a novel anti-inflammatory compound, 1,1-bis(3’-indolyl)-1-(p-chlorophenyl)methane or C-DIM12, derived from 3,3’-diindolylmethane (DIM), a naturally occurring condensation product of indole-3-carbinol. C-DIM12 has shown the ability in vitro to decrease glial-based inflammatory cytokines such as inducible nitric oxide synthase (iNOS), as well as TNFα. Additionally, the observed pharmacokinetics, oral bioavailability, and brain distribution of C-DIM12 prove appealing for therapeutic use to combat the progressive phase of neuron loss associated with PD. To this end, we postulated that oral delivery of C-DIM12 in a post-lesion MPTP and probenecid (MPTPp) model in adult transgenic (NF-κB-EGFP) mice would decrease dopamine neuron loss via suppression of glial activation. We further hypothesized that C-DIM12 inflammatory suppression is via a transrepressive mechanism, acting through nuclear receptors to clear NF-κB proteins from their respective pro-inflammatory gene promoters. Mice were treated with MPTPp for 7 days, followed by daily oral administration for of 50 mg/kg of C-DIM12 or corn oil (control) for one week. Animals given C-DIM12 showed significantly less glial and NF-κB activation, and an increase in dopamine neuron survival compared to animals receiving only corn oil. In conjunction, in vitro studies using BV-2 microglia cells and LPS indicated that C-DIM12 activates the NR4A2 (Nurr1) nuclear receptor to inhibit proinflammatory proteins such as iNOS and TNFα, and cytokines IL-1β and IL6. Based on these results, we concluded that C-DIM12 inhibits NF-κB-dependent gene expression by recruiting nuclear co-repressor proteins that inhibit transcription. When given orally, C-DIM12 inhibits neuroinflammation in vivo and therefore presents a promising small molecule platform for further pre-clinical investigation.

EXPERTISE: Immunofluorescence, cell culture, animal surgery, real time qPCR, immunoblot

32. Activation of the nuclear receptor Nur77 by a novel diindolylmethane analog suppresses inflammatory gene expression in 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 coordinate regulates the expression of multiple neuroinflammatory genes associated with PD including inducible nitric oxide synthase (iNos/Nos2), tumor necrosis factor alpha (Tnfa), and interleukin 1β (I1β). These observations suggest that inhibition of NF-κB could be a promising therapeutic target for preventing neuroinflammatory injury. Nuclear orphan receptors in the NR4A family, including NR4A1 (Nur77) and NR4A2 (Nurr1), have been shown to antagonize the effects of NF-κB on inflammatory gene expression. However, high affinity pharmacologic ligands of these receptors have been lacking. A novel ligand of NR4A1/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/EBP (p300), which is also required for the transcriptional activity of NF-kB. We therefore postulated 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). In the present studies, C-DIM5 increased expression of Nur77 mRNA and suppressed expression of the neuroinflammatory genes Nos2, IL-1β, and TNF-α. C-DIM5 also coordinately 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. In co-culture experiments, C-DIM5 protected neurons from apoptosis induced by activated glial cells. These data demonstrate that C-DIM5 prevents the production of neurotoxic inflammatory mediators in glial cells by inhibiting NF-κB.

EXPERTISE: Real time qPCR, PCR, tissue culture, western blotting, chromatin immunoprecipitation, immunofluorescence
33. Mice with genetic deficiency for complement receptor type 2 (CR2) show neuroprotection after experimental closed head injury.

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The pathophysiology of traumatic brain injury is characterized by complement activation, leading to neuroinflammation and delayed neuronal cell death. Complement receptor type 2 (CR2) has recently been identified as a “key player” in orchestrating complement-mediated immune responses. In the present study, we hypothesized that mice deficient in the CR2 gene (Cr2-/-) would be protected from complement-mediated secondary neuropathology after closed head injury. Adult C57BL/6 male Cr2-/- mice (n=98) and wild-type mice (n=157) were subjected to focal closed head injury, using a standardized weight-drop device. Sham-operated mice served as internal controls. Outcome parameters consisted of neurological scoring, quantification of inflammatory mediators in brain tissue and serum by Western blots and ELISA, assessment of glial activation and complement deposition in injured tissue by immunohistochemistry, and detection of neuronal cell death by TUNEL histochemistry. Head-injured Cr2-/- mice showed a significantly improved neurological outcome for up to 72 hours after trauma, compared to wild-type mice. While the post-injury release of pro- and anti-inflammatory cytokines was in a similar range between both groups, complement C3 deposition was markedly reduced in injured brain hemispheres of Cr2-/- mice. In addition, the activation of GFAP-positive astrocytes and CD11b-positive microglia was attenuated in head-injured Cr2-/- mice, compared to wild-type littermates. Cr2-/- mice also showed a decreased extent of neuronal cell death at seven days post-trauma by TUNEL histochemistry. These data emphasize a central role of CR2 in promoting complement deposition, glial activation, delayed neurodegeneration and adverse neurological outcome after closed head injury. Targeting complement activation on the level of CR2 may represent a promising future approach for therapeutic immunomodulation after closed head injury.

EXPERTISE: Animal handling (including, IP and IV injections, treatment dosage, anesthetic and analgesic administration and dosage), breeding colony management, behavioral testing, harvesting of tissues, tissue cryosectioning, tissue homogenization, serum and EDTA processing, immunohistochemical staining, western blotting, ELISA, scientific writing, grant proposal writing and organization.

34. Dysregulation of Rac of Rho induces death of motor neurons and activation of these GTPases is altered in the G93A mutant SOD1 mouse model of ALS

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Numerous studies have demonstrated a critical function for Rho GTPase family members (i.e., Rac, Rho, Cdc42) in neuronal development and survival. Although a pro-survival function for Rac has been reported in several neuronal cell types, the antagonistic relationship between Rac and Rho/ROCK signaling in neuronal survival remains poorly understood. In the current study, we examined the effects of targeted inhibition of Rac GTPase on motor neuronal survival. We demonstrate that treatment with NSC23766, a selective inhibitor of Rac GTPase, induces cell death of embryonic stem (ES) cell-derived motor neurons consistent with caspase-3 activation, dephosphorylation of AKT and ERK5, and nuclear translocation of the BH3-only protein Bad. We also examined the effects of a constitutive activator of Rho, CN03, on neuronal survival in vitro. In a manner similar to selective inhibition of Rac GTPase, treatment of ES cell-derived motor neurons with CN03 results in a marked loss of neurites and significant cell death. Interestingly, inclusion of the ROCK inhibitor Y-26732 was partially protective against either selective inhibition of Rac or constitutive activation of Rho in ES cell-derived motor neurons. These data suggest that the balance between Rac and Rho signaling is critical for motor neuron survival. Moreover, in the G93A mutant Cu,Zn-superoxide dismutase (SOD1) mouse model of amyotrophic lateral sclerosis (ALS), active Rac1-GTP immunoreactivity is markedly decreased in choline acetyltransferase (ChAT)-positive motor neurons of the lumbar spinal cord of end-stage mice when compared to age-matched wild type littermates. In addition, although immunoreactivity for total RhoB localizes principally to nuclei of ChAT-positive motor neurons from wild type mice, RhoB appears to redistribute to motor neuronal processes in end-stage mice harboring the G93A SOD1 mutation. Collectively, our data demonstrate that Rac and Rho are critical regulators of neuronal survival and as a result, disruptions in the balance of their activities may contribute to the etiology of motor neurodegenerative diseases such as ALS.

EXPERTISE: Cell culture, primary neuron isolation, enzyme assays, immunocytochemistry, immunohistochemistry, western blotting, immunoprecipitation, chromatin immunoprecipitation, animal handling,
tissue isolation and processing, cryosectioning, fluorescence microscopy, semi-quantitative and real time PCR, adenoviral purification and infection, bacterial transformations, propagation and culture of mouse embryonic stem cells, differentiation of mouse embryonic stem cell-derived motor neurons

35. Analysis of role of TRPV1 in kainic acid mediated toxicities: Evidence for a novel therapeutic intervention against epilepsy

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Transient receptor potential vanilloid 1 (TRPV1; capsaicin receptor) is a nonselective cationic channel protein expressed in the central and peripheral nervous system. Previous research demonstrates that capsaicin treatment decreased the seizures evoked by kainic acid (KA) in an animal model of temporal lobe epilepsy (Lee et al., 2011). This suggests a central role for TRPV1 in epilepsy. However, the mechanism by which KA activates TRPV1 to mediate epileptic seizures and how capsaicin prevents this remain to be investigated. Here we examined the hypothesis that KA causes Ca2+-dependent neurotoxicity via TRPV1 and that pretreatment with capsaicin or inhibition of TRPV1 activity by capsazepine (a TRPV1 inhibitor) prevents the effects of KA. In order to study the effects of KA on TRPV1, we used HEK293 cells that stably express TRPV1 (HEK TRPV1) to study KA-induced modulation of TRPV1. KA treatment activated Ca2+ into HEK TRPV1 cells but not in wild type HEK293 cells. Pretreatment of HEK TRPV1 cells with capsaicin (1 µM) or capsazepine (10 µM), significantly prevented the activation of TRPV1 by KA. Also, KA-mediated Ca2+ entry into HEK TRPV1 cells was suppressed by U73122 (PLC inhibitor). Therefore, we performed experiments to evaluate the effects of KA on PLC activation. KA treatment caused translocation of PH PLC delta-YFP from plasma membrane to cytosol suggesting the involvement of PLC in the effects of KA. Further, KA treatment caused actin condensation in HEK TRPV1 cells and capsaicin pretreatment prevented this. Collectively, our data suggest that KA causes cell toxicity via PLC dependent TRPV1 activation and cytoskeleton modifications and capsaicin pretreatment abrogates the effects of KA. We propose that desensitization or inhibition of TRPV1 is a novel therapeutic intervention against epilepsy.

EXPERTISE: Cell culture, calcium imaging, western blot analysis, immunoprecipitation, immunocytochemistry


FATEN I. TARAM, AN Winter, DA Linseman
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While the number of patients diagnosed with neurodegenerative disorders like Alzheimer’s disease, amyotrophic lateral sclerosis, and Parkinson’s disease are increasing, there are currently no effective treatments that significantly slow or halt the progression of these devastating diseases. Since oxidative and nitrosative stress are major factors in the underlying pathophysiology of these diseases, the identification of compounds that protect neurons from these stressors may lead to the development of novel therapeutic agents. Many natural compounds found in plants have significant intrinsic antioxidant properties. Chlorogenic acid (CGA), a polyphenolic compound that is abundant in coffee, and some of its metabolites are known to possess substantial antioxidant and free radical scavenging activity. In this study, we investigated the neuroprotective effects of CGA and its major metabolites in primary cultures of rat cerebellar granule neurons. We show that CGA and caffeic acid, which share a common catechol moiety, each displayed a dramatic protective effect against the nitric oxide donor, sodium nitroprusside. In marked contrast, ferulic acid and quinic acid, which lack the catechol structural motif, had no protective effect against nitrosative stress. Interestingly, the ability of these compounds to protect neurons against excitotoxicity induced by glutamate/glycine was completely independent of the presence of a catechol group. While CGA and quinic acid had no protective effect, caffeic acid and ferulic acid significantly protected neurons against glutamate-induced excitotoxicity. Moreover, caffeic acid was the only compound that displayed protective effects against hydrogen peroxide and the proteasome inhibitor PS341. Finally, none of the compounds tested showed any protective effect against caspase-dependent intrinsic apoptosis induced by removal of serum and depolarizing extracellular potassium (i.e., 5K apoptotic condition). We are currently testing the neuroprotective capacity of these compounds against other mechanistically distinct insults including endoplasmic reticulum stress. The aim of this study is to identify which structural motifs are responsible for the protective attributes of CGA and its metabolites against diverse types of neuronal injury.
37. β-Amyloid- and proinflammatory cytokine-induced cofillin-actin rod formation requires prion-dependent activation of NADPH oxidase.

KEIFER P WALSH¹, TB Kuhn¹,², LS Minamide¹, AE Shaw¹, J Cichon³, W-B Gan³, J Mi¹, BW Bernstein¹, JD Lambeth⁴, DR Brown⁵, MD Zabel⁶, JR Bamburg¹.


Persistent exposure of neurons to hypoxia/ischemia, excitotoxic glutamate, and β-amyloid peptide (Aβ) all provoke a remodeling of the neuronal actin cytoskeleton into rod-like cofillin-saturated actin bundles (rods). Rods disrupt synaptic function by blocking transport and/or sequestering cofillin from dendritic spines. These inducers generate reactive oxygen species (ROS) and rod formation requires cofillin oxidation to form an intermolecular disulfide bond. Here we show that proinflammatory cytokines (e.g. TNFα) induce rods in the same population (~20%) of hippocampal neurons that respond to SDS-stable Aβ dimer/trimer (Aβd/t), a physiologically relevant species in Alzheimer's disease (AD). Neurons lacking the cellular prion protein (PrPc) do not form rods in response to Aβd/t or TNFα, but do upon treatment with glutamate or mitochondrial inhibitors, suggesting these latter inducers utilize a different pathway. This finding was confirmed by inhibiting NADPH oxidase (NOX) isoforms 1 and 2 by expressing dominant interfering gp22PHOX or by using pharmacological inhibitors, each of which blocked rod formation in response to Aβd/t and TNFα. Because cognitive impairment in Aβ-overproducing AD mice is also PrPc-dependent, we suggest rod formation mediates loss in synaptic plasticity. To study rods and their impact on neural circuit function in vivo, we have developed adeno-associated viruses to visualize fluorescent cofillin signals in dendrites of pyramidal neurons using two-photon microscopy. Local treatment with endothelin-1, a potent vasoconstrictor, induces significant rod pathology within hours and was accompanied by deficits in treadmill running behavior monitored during imaging. Cofilin-actin rods could explain the common pathologies of familial and sporadic AD, as well as synaptic dysfunction in AD, stroke, and other neurological disorders.

38. Differential neuroprotective effects of anthocyanins and their metabolites against nitrosative stress

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From The Department of Biological Sciences, University of Denver, Denver, CO

Evidence implicates oxidative and nitrosative stress as underlying causes of the neuronal death observed in diverse neurodegenerative diseases. Supplementation of antioxidant defenses may be an effective therapeutic strategy for diseases such as amyotrophic lateral sclerosis, Parkinson’s disease, and Alzheimer’s disease. In this context, nutraceutical antioxidants have provided promising insight into prospective treatment strategies; however, a number of nutraceuticals are protective against only the reactive oxygen species (ROS) associated with oxidative stress, and lack the capacity to defend neurons from equally damaging reactive nitrogen species (RNS). In this study, we explore the capacity of two anthocyanin-enriched extracts from strawberries (SAE) and blackberries (BAE), their respective principle anthocyanin constituents, callistephin (pelargonidin-3-glucoside) and kuromanin (cyanidin-3-glucoside), and their respective metabolites, 4-hydroxybenzoic acid (HBA) and protocatechuic acid (PCA), to protect cerebellar granule neurons (CGNs) from damage induced by either oxidative or nitrosative stress. While all of the extracts and pure compounds tested in this study protected CGNs from oxidative stress induced by either glutamate excitotoxicity of hydrogen peroxide, a stark contrast was observed under conditions of nitrosative stress, as only BAE, its primary anthocyanin, kuromanin, and its metabolite, PCA, displayed the capacity to defend neurons from nitric oxide (NO)-induced apoptosis. Strikingly, the protective effect of kuromanin in CGNs was blocked by the addition of polyethyleneglycol-Cu/Zn superoxide dismutase (PEG-SOD), suggesting a dependence upon superoxide. Kuromanin protection of Neuro2A neuroblastoma cells from NO-induced cell death was prevented by overexpression of SOD1. Based on these observations, we suggest a unique mechanism by which slight structural variances, specifically, the absence or presence of a catechol moiety, lend kuromanin and its metabolite the unique ability to generate superoxide which acts as a scavenger of NO. These findings indicate that kuromanin or PCA may be more effective therapeutic agents in neurodegenerative diseases that involve significant RNS generation in addition to ROS involvement than either callistephin or HBA, which lack a catechol moiety.
39. Role of Mammalian STE20-like Kinase 4 (MST4) in Pituitary Tumorigenesis
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Gonadotrope or nonfunctioning pituitary tumors often present with signs of hypogonadism together with optic chiasm compression and surgery and radiation are the only therapeutic options. To identify dysregulated genes and pathways that may contribute to gonadotrope tumorigenesis, we performed DNA microarrays on 14 pituitary tumors, 9 normal human pituitaries and copy number variation arrays on 10 tumors using a combined genetic and genomic screen. We identified one tumor with deletion of the X chromosome other than a small amplification including the transcript encoding for Mammalian Sterile 20 like kinase 4 (MST4). MST4 mRNA and protein was consistently upregulated in tumors and absent in normal pituitary. MST4 stable transfectants resulted in increased colony formation in soft agar and increased rates of cell proliferation, dependent on the p38MAPK and PI3K/Akt pathways. MST4 decreased susceptibility to apoptosis in response to hypoxia as assessed by caspase-3 cleavage and nuclear condensation, dependent on the kinase domain. Hypoxia-induced factor (HIF) 1 was upregulated in MST4 transfectants. Inactivation of HIF1 abolished the protective effects of MST4. MST4 is a novel pituitary tumor oncogene whose expression promotes tumorigenesis via dual effects on proliferation and survival under hypoxic stress and the first kinase identified that may be targetable.

EXPERTISE: Real-time PCR, immunoblot, immunocytochemistry

Neural Excitability, Synapse and Glia

40. Single particle tracking of Nav1.6 molecules demonstrates different mechanisms for sodium channel anchoring within the AIS versus the soma of hippocampal neurons.
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Voltage-gated sodium channels are responsible for the initiation of action potentials in excitable cells. These channels are highly concentrated at the axon initial segment (AIS) of neurons due to their interactions with Ankyrin-G. This interaction is mediated by a 9 amino acid sequence, termed the Ankyrin Binding Motif (ABM) present on the intracellular loop between domain II and domain III. In order to study the dynamics of sodium channels in living neurons in real time, we created a fluorescently tagged Nav1.6 protein with an extracellular tag (biotin acceptor domain). We used single molecule tracking of channels labeled with streptavidin conjugated Quantum Dots (QDs) and/or Alexa594 to measure the mobility of Nav1.6 channels localized to the AIS and somatodendritic compartments of 6-14 DIV hippocampal neurons. The tracks revealed that somatic Nav1.6 channels show periods of transient confinement. To test the hypothesis that this behavior is influenced by interactions with Ankyrin-G, we deleted the ABM from the Nav1.6 construct. This mutant channel does not concentrate at the AIS and instead localizes throughout the soma and processes as expected, based on both GFP fluorescence and labeling of surface channels using streptavidin conjugated-Alexa594. However, surprisingly, single particle tracking of the mutant channels revealed that this channel also transiently binds within discrete regions on the soma. This suggests that although ankyrin-G binding is necessary and sufficient for Nav1.6 to localize to the AIS, another mechanism is responsible for the localization and membrane dynamics in the somatodendritic region of neurons.

EXPERTISE: Molecular biology, confocal and TIRF microscopy, electrophysiology

41. A casein kinase 1 delta mutation in familial migraine with aura and sleep disorder.
EMILY BATES, KC Brennan, R Shapiro, A Charles, L Ptacek.
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Though migraine is a common disorder, its mechanisms are poorly understood. We identified two families with mendelian inheritance of migraine with aura and circadian dysfunction. In one family, a mutation was identified in the casein kinase 1 δ gene that caused a T44A substitution within the conserved kinase domain. The second family had a mutation encoding a H46R change two amino acids away. Both mutations lead to reduced kinase function. When the CK1 δ-T44A allele is expressed in mice, mice recapitulated a circadian phenotype. To ascertain whether this mutation also conferred characteristics associated with migraine, we recorded threshold for cortical spreading depression, the event thought to be the physiological correlate of the migraine aura. We found a significantly reduced threshold for CSD in CK1 δ-T44A mutant mice, consistent with
increased cortical excitability. We also observed larger vascular changes associated with CSD, suggesting a modification of neurovascular coupling. Next we investigated the response to nitroglycerin, a commonly used model of migraine nociception. CK1 δ mutant mice showed reduced thermal and mechanical nociceptive thresholds compared to wild type siblings. This suggests an alteration in nociceptive processing in CK1 δ-T44A mice. Finally, we examined the stimulus response of neuronal and astrocytic cultures in mutant and wild type mice. No difference was seen in neuronal responses to NMDA or elevated K+, however in astrocytes we identified an increased spontaneous and evoke CA+ signaling in CK1 δ-T44A mice, suggesting a difference in astrocyte excitability.

42. Wheel running produces widespread structural changes in striatal and limbic regions involved in stress.


From the Department of Integrative Physiology & Center for Neuroscience, University of Colorado Boulder. Exercise increases resistance against stress-related disorders such as depression and anxiety, but the mechanisms remain unknown. Exercise produces neuro-plastic and functional adaptations within a variety of brain regions implicated in the behavioral consequences of stress. In the hippocampus, for example, wheel running produces increases in dendritic complexity that parallel improvements in contextual and spatial memory. The effects of exercise on neuronal structure within other brain regions affected by stress, however, remain unexplored. Among the behaviors impacted by stress that are prevented by prior exercise are deficits in instrumental learning (putatively involving the dorsal striatum). We examined, therefore, the effects of voluntary wheel running on dendritic arborization in the dorsomedial and dorsolateral striatum. Adult, male Fisher (F344) rats (n=7/grp for hippocampal analysis and n=3/grp for striatum analyses) were allowed either voluntary access to running wheels or remained sedentary for 6 weeks. Brains were immediately removed and processed in accordance with Golgi Stain protocols. Sholl analysis was used to quantify pyramidal cells with the CA3 region of the hippocampus and medium spiny neurons within the dorsal striatum. Wheel running increased dendritic complexity within the CA3 region of the hippocampus as well as within the dorsal striatum. These data suggest that exercise produces extensive alterations in neuronal structure that may be involved in stress resistance.

EXPERTISE: golgi

43. Optical detection of glutamate transients within olfactory bulb glomeruli.

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Glomeruli in the olfactory bulb are discrete functional units important for the processing of olfactory information. Within each glomerulus, dendritic tufts of mitral and tufted cells receive synaptic input from a convergence of olfactory sensory nerves (OSNs) expressing a single odorant receptor. In addition to these axodendritic synapses, dendrodendritic synapses and extrasynaptic signaling all play a role in shaping the output of glomeruli to higher order processing areas in the cortex. However, the precise spatial and temporal dynamics of glutamate in a glomerulus is not known, nor the factors that constrain these dynamics. We used a protein sensor (FLII81E-1µ) that reduces fluorescence (Förster) resonance energy transfer (FRET) upon binding of glutamate to examine directly glutamate transients in a glomerulus. Following dye-loading (concentration = 50 ng/ml) in rat bulb slices, FRET signals (CFP excitation at ~440 nm; Venus (Y) emission at ~535 nm) were visualized in the superficial 50 µm of the slice using an upright fluorescence microscope (Zeiss Axioskop) equipped with DIC optics. Electrical stimulation of OSNs (<100 µA; 100 µsec) resulted in a decrease in the FRET signal within glomeruli (ΔFY ~ 1.1%; n = 47 glomeruli, 15 slices) that persisted for ~500 ms. FRET signals were largest in the center of glomeruli and diminished with distance from the glomerular layer (n = 11 glomeruli, 10 slices). Bath application of the glutamate uptake inhibitor dl-threo-b-benzyloxyaspartate (TBOA, 25 µM) prolonged the FRET signals (half-width ~ 1000 ms, n = 6 glomeruli, 3 slices) but the spatial dynamics were unchanged. In addition, depolarization of an external tufted cell at a glomerulus with a patch electrode (70 mV; 50 ms) was sufficient to elicit FRET signals (n = 3), suggesting that the sensor is sufficiently sensitive to detect glutamate transients elicited by stimulation of one cell. Our results suggest that FLII81E-1µ is a sensitive detector of glutamate in olfactory bulb glomeruli and that the spread of extrasynaptic glutamate is mediated by neural/glial processes rather than the action of transporters.

EXPERTISE: physiology, electron microscopy
44. Mitochondrial Control of Angiotensin Signaling in Cerebral Vascular Smooth Muscle.

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Reactive oxygen species (ROS) have been shown to be involved in the pathogenesis of vascular disease. Previously, we have shown that endogenous ROS modifies L-type calcium channel signaling in murine vascular smooth muscle cells (VSMC), and that this process is locally regulated by angiotensin II (Ang) via activation of membrane-bound NAD(P)H oxidase. Mitochondria serve as another endogenous source of ROS and have been shown to have both ROS and calcium sensitivity. We therefore decided to test the hypothesis that mitochondria are involved in local Ang signaling in murine cerebral VSMC. Using a combination of Total Internal Reflection Fluorescence Microscopy (TIRFM) and confocal imaging, we were able to determine a close spatial relationship between a subpopulation of mitochondria and the sarcolemma in isolated myocytes. Significant coupling occurred between these mitochondria and Ang-induced localized calcium influx events (sparklets). Using either the electron-transport chain (ETC) complex I inhibitor, rotenone, or the mitochondrial-targeted superoxide scavenger, mitoTEMPO, we were able to inhibit the ability of Ang to induce persistent calcium sparklets. Conversely, by uncoupling ETC complex III using antimycin-A, we were able to stimulate ROS-dependent persistent sparklet activity and sarcolemmal oxidative puncta formation. Additionally, we were able to significantly diminish Ang-induced vasoconstriction in pressurized, isolated cerebral resistance arteries, by bath application of mitoTEMPO. From these results, we conclude that sub-sarcolemmal mitochondria participate in local Ang signaling and mediate Ang-induced vasoconstriction in murine cerebral resistance arteries.

EXPERTISE: calcium imaging, live cell imaging, pressurized artery experiments, TIRF microscopy, confocal microscopy

45. A Large Field-of-View Nonlinear Microscope for Biological Imaging.

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From the Department of Electrical and Computer Engineering, Colorado State University, Fort Collins, CO; Department of Physics, Colorado School of Mines, Golden, CO; Department of Biomedical Sciences, Colorado State University, Fort Collins, CO; School of Biomedical Engineering, Colorado State University, Fort Collins, CO; and Department of Chemistry, Colorado State University, Fort Collins, CO.

Multiphoton laser scanning microscopy (MPLSM) has become a standard tool for high fidelity imaging of biological tissues. Numerous in vivo and in vitro imaging systems have been exploited to study structure and function in biological specimens. Biological processes occur on numerous spatial scales, making it ideal to collect data on varied spatial scales simultaneously. While the typical MPLSM imaging system can obtain images over large regions (~1 square-mm), this is often achieved by use of low magnification objective lenses with a reduced numerical aperture (NA). Since the lateral spatial resolution of a multiphoton microscope is linearly proportional to the NA of the excitation objective, a large field of view (FOV) is often imaged at the expense of spatial resolution. This can be detrimental to data interpretation if small-scale and large-scale biological processes are to be studied in tandem. Here we present a new MPLSM system that is capable of generating images over a large FOV without low magnification objectives. The efficiency of nonlinear contrast excitation is improved by use of commercially available telecentric scan lenses, while collection efficiency is enhanced by utilizing a custom objective lens. We rigorously characterize the properties of both excitation and collection for this imaging system, and present characteristic images of nonlinear contrast signals from biological media.

EXPERTISE: Imaging

46. Endoplasmic reticulum-plasma membrane contacts function as membrane protein trafficking hubs at the neuronal soma.

PHILIP D FOX, CJ Haberkorn, AV Wiegel, EJ Akin, MJ Kennedy, D Krapf, MM Tamkun.
From the Department of Biomedical Sciences, the School of Biomedical Engineering and the Department of Electrical and Computer Engineering, Colorado State University, Fort Collins, CO.

Endoplasmic reticulum/plasma membrane (ER/PM) contacts are a specialized subcellular structure where the two lipid bilayers are held together by direct protein-protein or protein-lipid interactions. We provide evidence for a novel role of ER/PM contacts as trafficking hubs for insertion and removal of plasma membrane proteins in HEK cells and neurons. By simultaneously visualizing ER/PM contacts and various transmembrane protein cargoes with total internal reflectance (TIRF) microscopy, we demonstrate that the vast majority of exocytotic
47. Aβ42 Induces Neuronal Hyperexcitability in a Pathway that Leads to Increased Seizure Sensitivity, Neurodegeneration, Motor Deficits, and a Shorter Lifespan.

Y Ping, EU-TEUM HAHM, GIRMA WARO, D Vo-Ba, and S Tsunoda.

From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO

The accumulation of β-amyloid (Aβ) peptides is an early step in the pathogenesis of Alzheimer’s disease (AD), and has recently been suggested to lead to neuronal hyperexcitability in mammals. The underlying intrinsic changes, as well as the downstream consequences of this increased excitability, are unknown. Here, we use a transgenic Drosophila line that expresses human Aβ42; this model has been shown to exhibit age-dependent learning and locomotor defects, progressive neurodegeneration and a shortened lifespan. We show that Aβ42 induces hyperexcitability and an increase in seizure sensitivity. To investigate the potential underlying intrinsic changes generated by Aβ42, we recorded from neurons in culture, as well as in the intact brain. We show that Aβ42 induces a decrease in the A-type K+ current encoded by Kv4. These results are consistent with our previous studies which have shown that a loss in Kv4 channel function leads to increased excitability. Additionally, we show that the Aβ42-induced decrease in IA is due to degradation of Kv4 channels via a proteasome-dependent pathway. To identify downstream consequences of the Aβ42-induced loss of Kv4 channels, we transgenically expressed Kv4 to reach near wild-type protein levels, and then tested for rescue of downstream phenotypes induced by Aβ42. We show that restoration of Kv4 in Aβ42-expressing flies significantly attenuates the increase in seizure sensitivity, neurodegeneration, and locomotor effects induced by Aβ42 expression alone. Expression of Kv4 also restores a near-normal lifespan to Aβ42-expressing flies. Altogether, these results suggest that hyperexcitability, generated significantly by down-regulation of Kv4 channels, plays a key role in the Aβ42-induced pathway leading to decreased seizure threshold, neurodegeneration, motor deficits, and shortened lifespan.

EXPERTISE: electrophysiology, immunocytochemistry, immunoblot analyses, Drosophila genetics

48. Effects of dopamine on frequency dependent synaptic transmission.

JONNA M. LEYRER, MP Thomas.

From the School of Biological Sciences, University of Northern Colorado.

In humans, prefrontal cortical areas are known to support spatial and object-related working memory (WM) processes. In mice, working memory is mediated by homologous regions in medial prefrontal cortex (mPFC). Patients with schizophrenia often suffer deficits in WM, and often show abnormal rhythm generation within the prefrontal cortex. While it is well established that WM tasks are critically dependent on optimal levels of dopamine in the PFC, the cellular mechanisms of dopamine actions regulating WM are currently unknown. Rhythmic activity has been hypothesized to play an essential role in memory encoding and synchronizing network activity. Rhythm generation in cortical circuits likely involves an interaction between intrinsic electrical properties of neurons and frequency-dependent synaptic properties. Pathological changes in rhythmic activity accompany WM deficits in schizophrenic patients, supporting the hypothesis that oscillations play an important role in mnemonic processes and that disruption of rhythm generation is directly related to the SZ phenotype. Working memory processes depend on stable patterns of neuronal activity, which may be sensitive to frequency changes in inhibitory and excitatory transmission. We aimed to determine the effects of dopamine on frequency dependent short-term synaptic plasticity (10Hz-50Hz) in layer V pyramidal neurons of the mPFC in the mouse. Whole cell patch clamp recordings were performed from layer V pyramidal neurons in coronal slices of mouse mPFC. To study short term synaptic plasticity, stimulating electrodes were placed in layer V to activate primarily fibers from other layer V pyramids. EPSPs were evoked in Iclamp 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 the presence of APV to isolate AMPA receptor-mediated EPSPs. We aimed to determine if cells were facilitating (i.e. the second EPSP was greater in amplitude than the first) or depressing (i.e. the second EPSP was smaller in amplitude than the first). The effects of dopamine receptor stimulation on AMPA currents may provide a mechanism for dopaminergic modulation of rhythm generation in prefrontal cortical circuits.

EXPERTISE: Electrophysiology; Immunohistochemistry
49. Mu-opioid and GABAB receptor mediated inhibition of GABA release onto POMC neurons occurs through a mechanism that is Ca2+-independent and resistant to desensitization.

REAGAN L. PENNOCK, ST Hentges.

From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.

GABA release onto proopiomelanocortin (POMC) neurons of the arcuate nucleus is robustly inhibited by the activation of presynaptic mu-opioid (MOR) and GABAB (GABABR) receptors. MORs and most GABABRs maintain maximal inhibition of GABA release during a prolonged exposure to an agonist, demonstrating resistance to acute desensitization. However, in a subset of terminals onto POMC neurons GABABR-mediated inhibition of GABA release diminishes during a prolonged exposure to an agonist. We hypothesize that GABABR-mediated signaling that diminishes during a constant exposure to an agonist may result from inactivation of the effectors this subset of receptors couples to. Whole-cell voltage clamp recordings were made from POMC neurons to determine whether the disruption of voltage-dependent Ca2+ channel (VDCC) or voltage-dependent K+ channel (VDKC) signaling affect the ability of agonists of the MOR (DAMGO) or GABABR (baclofen) to induce the inhibition of GABA-mediated postsynaptic currents (IPSCs). The presence of VDKC blockers did not inhibit the ability of either DAMGO or baclofen to induce inhibition of IPSCs. DAMGO and baclofen induced inhibition was also unaffected when recordings were made in a Ca2+-free external recording solution, suggesting that inhibition of Ca2+ influx is unnecessary for either MOR- or GABABR-mediated inhibition of release. To confirm that inhibition of release may occur in a manner independent of Ca2+ influx, the ability of DAMGO and baclofen to induce inhibition of GABA release was measured in the presence of the Ca2+ ionophore ionomycin. Ionomycin robustly increased IPSC frequency (3 fold) but did not occlude either DAMGO or baclofen induced inhibition of release. Post hoc analysis detected no subset of recordings in which GABABR-mediated inhibition of release was occluded by any of the above treatments. These data demonstrate that diminished GABABR-mediated signaling in a subset of terminals onto POMC neurons is unlikely the result of differential receptor-effector coupling, and that MOR- and GABABR-mediated inhibition of GABA release onto POMC neurons likely occurs through a Ca2+-independent mechanism.

EXPERTISE: Electrophysiology, Live brain slice preparation

50. The Role of the C2A Domain of Synaptotagmin in Asynchronous Release.

MALLORY SHIELDS, N Reist.

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Understanding the mechanisms mediating information transmission across a chemical synapse is essential to understanding brain function. During synaptic transmission, membranous vesicles within neurons are loaded with neurotransmitter, docked to the presynaptic cell membrane, primed for release, and fused with the presynaptic membrane to release neurotransmitter onto the next, or postsynaptic, cell. The vesicle membrane and proteins are recycled back into the presynaptic cell to be utilized later. There are three types of neurotransmitter release, two of which are Ca2+-dependent: fast, synchronous release, and a more prolonged, asynchronous release. Asynchronous release has recently been proposed to play a role in synaptic plasticity, the basis for learning and memory mechanisms. These release processes are tightly regulated by a number of key synaptic proteins, including Ca2+ sensors and the fusion machinery complex. One such protein, synaptotagmin, is proposed to be the low-affinity Ca2+ sensor that, upon Ca2+ binding, triggers fast, synchronous release of neurotransmitter. In addition, studies have shown interplay between synaptotagmin and a currently unidentified and controversial high-affinity Ca2+ sensor responsible for the prolonged, asynchronous neurotransmitter release mechanism. At this time, it is postulated that synaptotagmin is directly inhibiting the asynchronous Ca2+ sensor. However, what this interplay entails is currently unknown and poorly understood. Using specific point mutations in vivo, the role of synaptotagmin in modulating asynchronous release will be investigated.

51. Activation of serotonin-2a receptors induces rhythmic oscillatory bursting in layer 5 pyramidal neurons of the mouse medial prefrontal cortex.

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Using whole cell patch-clamp recordings obtained from acute coronal sections of 28-36 day old C57bl/6 mice, we have recently discovered that bath application of the selective 5-HT2a agonist TCB-2 elicits rhythmic oscillatory bursting (ROB) in type I, but not type II layer 5 pyramidal neurons of the mPFC after 15-20 seconds of regular spiking evoked by a 60 second DC current injection. Arrhythmic burst firing was observed in the presence of the GABA antagonist bicuculine (10uM), and ROB was observed only in the presence of both
bicuculine and the AMPA channel antagonist DNQX (20uM). Under these conditions, burst discharge was defined as relatively high rates of action potential firing (12-15 spikes/sec) separated by a refractory inter-burst period (700-1000ms). The minimal amplitude of the interspike intervals (ISImin) varied between intra-burst and inter-burst periods with inter-burst interspike intervals exhibiting substantially greater ISImin values. Analysis of burst discharge was carried out using custom scripts written for MATLAB in which the coefficient of variation (Cv) of the ISImin was determined. Since ROB was observed after a period of regular spiking, the Cv of the ISImin was calculated throughout traces based on a 4-point moving average. The Cv of 60 second induced spike trains was significantly greater at all times after 20 seconds in neurons superfused with TCB-2, bicuculine, and DNQX than control traces, or traces recorded in the presence of bicuculine and DNQX. Inhibitory post-synaptic potentials (IPSPs) were observed in traces recorded in cells where TCB-2 was applied without bicuculine. Cv of ISImin was significantly lower in the 6 spikes following IPSPs (Mean=.0163) than the 6 spikes preceding them (Mean=.033, p<.005). These results indicate that activation of 5-HT2a receptors is capable of inducing burst-firing in type I layer 5 pyramidal neurons of the mPFC, but that this firing mode is generally suppressed by GABAergic interneurons, and the rhythmicity of the bursts can be destabilized by glutamatergic inputs.

EXPERTISE: Whole cell patch-clamp physiology, immunohistochemistry

Neuroendocrine

52. Effects of repeated voluntary or forced exercise on rat brain thermosensitive serotonergic systems

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Exposure of rats to elevated ambient temperature activates subpopulations of brainstem serotonergic neurons in the dorsal raphe nucleus (DR), the main source of serotonergic innervation of the forebrain. Thermosensitive subpopulations of serotonergic neurons include those in the ventrolateral part/ventrolateral periaqueductal gray (DRVL/VLPAG) and those in the interfascicular part of the dorsal raphe nucleus (DRI). DRVL/VLPAG serotonergic neurons are thought to be sympathomotor command neurons involved in inhibition of sympathetic outflow, while DRI serotonergic neurons project to forebrain areas involved in cognitive function and mood, such as the prefrontal cortex and hippocampus, and are thought to be involved in stress resilience. Prior studies have shown that either voluntary or forced wheel running for six weeks has stress resistance effects in rodent models, effects that are dependent on alterations in serotonergic function. It is unclear how voluntary exercise is associated with activation of the DRVL/VLPAG and DRI serotonergic neurons and how this activation is associated with the stress resilient effects of voluntary exercise. Considering these outcomes, the objective of the current study was to determine if voluntary or forced wheel running activates DRVL/VLPAG and DRI serotonergic neurons. To investigate this question we conducted two studies. The first study examined expression of the protein product of the immediate-early gene, c-fos, and tryptophan hydroxylase (TPH) using immunohistochemistry in rats exposed to either 1) chronic voluntary exercise for 6 weeks or 2) sedentary control conditions for 6 weeks. The second study included a third treatment group in which rats were exposed to forced exercise for 6 weeks. In Study 1, rats exposed to repeated voluntary exercise had increased c-Fos expression in non-serotonergic neurons within the DRI, relative to sedentary controls. In Study 2, we replicated the findings in Study 1. In addition, rats exposed to repeated forced exercise, but not repeated voluntary exercise, had increased c-Fos expression in serotonergic neurons in the DRI. These results suggest that repeated forced exercise, but not repeated voluntary exercise, activates DRI serotonergic neurons, an effect that may contribute to the recently described stress resilience effects of forced exercise. These results also suggest that the stress resistance effects of chronic voluntary and forced exercise may involve different mechanisms.

53. The presence of endogenous glucocorticoids differentially influences acute stress-induced clock gene expression in extra-SCN regions of the rat brain.

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Cellular clocks depend on molecular oscillators derived from counterregulatory expression of clock genes. Disrupted clock gene expression has been linked to a variety of mood disorders (e.g. bipolar disorder and depression). Rhythmic expression of these clock genes is found within the body’s master clock, the suprachiasmatic nucleus (SCN), as well as extra-SCN brain areas. In addition to the circadian rhythm of clock genes, there is a diurnal rhythm of corticosterone (CORT) release. Most brain areas and peripheral tissues,
with the SCN as a notable exception, contain glucocorticoid receptors. A core clock gene, Per1, contains a functional glucocorticoid response element within its promoter region, making Per1 transcription susceptible to circulating CORT levels. We hypothesize that the SCN coordinates clock gene expression throughout the brain and body at least in part by the diurnal release of CORT. Thus, our present study examined the effect that removal of endogenous CORT via adrenalectomy (ADX) ± 30 min of acute restraint stress at different times of day (ZT4 & 16) has on Per1 and Bmal1 mRNA expression within the SCN, paraventricular nucleus (PVN) and medial prefrontal cortex (mPFC) in male rats maintained on a 12:12 h LD cycle. ZT4 & 16 have different levels of Per1 and Bmal1 mRNA in the SCN and PVN, and to a lesser extent mPFC, which we replicated in our adrenal-intact rats. Using in situ hybridization, we found that acute stress increased Per1 but not Bmal1 mRNA in the PVN at both ZTs. This effect was dependent on the presence of endogenous CORT. Stress had a less robust effect within the mPFC, which did not depend on the presence of CORT. Stress or ADX had no effect on diurnal Per1 or Bmal1 mRNA in the SCN. ADX alone ablated the diurnal difference seen in both Per1 and Bmal1 mRNA levels in the PVN, suggesting that basal, endogenous CORT is necessary for normal Per1 and Bmal1 expression in the PVN. There are sex differences in stress reactivity. Thus, we compared the effect of acute stress on clock gene expression in adrenal-intact female and male rats in the SCN & PVN at ZT4 & 16; results replicated the first experiment and no sex difference was found.

EXPERTISE: In situ hybridization

54. Actin cytoskeleton modulates local L-type calcium channel signaling and ERK activation 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 enhanced luteinizing hormone (LH) biosynthesis and secretion. This process is essential for follicular maturation and ovulation. Previous research suggests that the extracellular signal regulated kinase (ERK) is activated by Ca2+ influx through L-type Ca2+ channels to increase LH synthesis. What is missing, however, is direct demonstration of localized Ca2+ influx at the plasma membrane and how the spatiotemporal dynamics of these channels affect downstream signaling. We hypothesize that disrupting actin cytoskeleton, which is important for membrane organization and cell remodeling, will affect localized GnRH-induced Ca2+ influx and ERK activation in gonadotropes. To test this hypothesis, we used a combination of TIRF microscopy and electrophysiology to image subplasmalemmal Ca2+ influx in the gonadotrope cell line alphaT3-1. Using this approach we visualized discrete sites of Ca2+ influx (called “Ca2+ sparklets”) which produced microdomains of elevated Ca2+ on the cell surface. Pretreatment of alphaT3-1 cells with Jasplakinolide (Jas; 100nM), a pharmacological disruptor of actin cytoskeleton, decreased the Ca2+ influx at GnRH (3nM) induced Ca2+ sparklets sites, but did not change the number of sites on the plasma membrane. Jas did not affect Ca2+ sparklet activity or density induced by PKC agonist phorbol 12, 13-dibutyrate (PDBu; 50 nM) or L-type Ca2+ channel agonist FPL64176 (500 nM). Therefore, actin disruption with Jas interrupts GnRH signaling and ERK activation by decreasing the Ca2+ influx through L-type Ca2+ channels, but does not affect Ca2+ activity or ERK activation downstream of GnRH when activating PKC or L-type Ca2+ channels directly. In summary, these data indicate that in alphaT3-1 cells, GnRH engages the actin cytoskeleton for localized Ca2+ influx through L-type Ca2+ channels and ERK phosphorylation, and demonstrates the specificity in spatial and temporal organization to propagate extracellular signals into intracellular signals for LH biosynthesis.
EXPERTISE: calcium imaging, electrophysiology, tissue culture

55. Chronic glucocorticoid intake alters basal Tph2 protein expression in anxiety- and resilience-related midbrain serotonergic systems.

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From the Department of Integrative Physiology and Center for Neuroscience, University of Colorado, Boulder, CO.
Previous research from our lab demonstrated in rats that chronic glucocorticoid (GC) intake via the drinking water dose-dependently induces an anxiety- and depressive-like behavioral phenotype, and disrupts the diurnal expression pattern of tph2, the gene encoding the rate-limiting enzyme for brain serotonin synthesis (tryptophan hydroxylase 2, Tph2). Chronic GC treatment has furthermore been shown to potentiate basal and stress-induced Tph activity in anxiety-related subdivisions of the midbrain dorsal raphe nucleus (DR). Here, we used western blot technique to test the hypothesis that chronic GC treatment also increases basal Tph2
protein expression. Adrenal-intact, adult male rats were treated with 100 µg/ml corticosterone (CORT) or vehicle (0.45% 2-hydroxypropyl-β-cyclodextrin) via the drinking water for 21 days, and their emotional behavior was assessed in the elevated plus-maze (EPM) and forced swim tests (FST) after 2 weeks. In concordance with previous studies, chronically GC-treated rats displayed more anxiety-like behavior on the EPM, and in tendency less proactive stress-coping behavior in the FST. In GC-treated rats, light-phase Tph2 protein expression was significantly elevated in anxiety-related subdivisions of the DR, namely the ventral (DRV) and dorsal part (DRD). Dark-phase Tph2 expression in the DR remained unaltered by GC treatment, while dark-phase (but not light-phase) Tph2 expression in the resilience-related median raphe nucleus (MnR) was in tendency (p=0.063) decreased after chronic GC exposure. Our results suggest that anxiety- and resilience-related brain serotonergic systems in the DR and MnR, respectively, are particularly sensitive to GC-induced alterations of Tph2 protein expression, with anxiety-related DR subdivisions being disrupted during the inactive light-phase, while the resilience-related MnR system appears to be affected during the active dark-phase. It remains to be determined whether these region-dependent effects of chronic GC exposure on Tph2 protein expression result in altered basal and stress-induced serotonin release in specific anxiety-and resilience-related fore- and hindbrain target regions.

EXPERTISE: in situ hybridization, western blot, immunohistochemistry, behavioral testing (anxiety, depressive-like behavior), adrenal functionality assay

56. Identifying mechanisms by which exercise prevents inescapable stress induced instrumental learning deficits

PJ Clark¹, J Amat², PARSA R. GHASEM³, SO McConnell¹, SF Maier², BN Greenwood¹, M Fleschner¹.

From the ¹Department of Integrative Physiology & ²Center for Neuroscience, University of Colorado Boulder. The World Health Organization predicts depression will become the largest economic health burden on society by 2030. However, the neural mechanisms that underlie depression are not well understood. Exposure to uncontrollable stress is a major risk factor for developing mood disorders like depression. In rat models, exposure to inescapable stress (IS) produces a sequelae of depression-like behaviors including deficits in the shuttle box escape task, a form of instrumental learning. Shuttle box escape deficits following IS are dependent on sensitized serotonin (5-HT) activity at receptors in the dorsal striatum during the mild stress (foot shock) used to motivate task acquisition. Our lab has observed that shuttle box escape deficits following IS are prevented in rats that engage in voluntary wheel running for 6 weeks prior to IS. Yet, comparatively less is known about how exercise prevents such deficits. One hypothesis is that exercise attenuates IS-sensitized 5-HT transmission in the dorsal striatum during mild stress, which may consequently impact activity of additional neurotransmitters critical for instrumental learning including dopamine (DA). Attenuated 5-HT and/or potentiated DA transmission in the striatum of running animals during stress could provide protection against shuttle box deficits. The purpose of this experiment was to identify the effects of mild stress (two foot shocks, 0.8mA, 5s duration) applied 24h following IS on extracellular concentrations of 5-HT and DA in the dorsal medial and lateral striatum of running and sedentary adult male Fischer 344 rats using in vivo microdialysis.

EXPERTISE: immunohistochemistry, in situ hybridization

57. Glucocorticoid-dependent dynamic modulation of sgk1 gene expression within oligodendrocyte dense white matter of rat brain in response to acute stress and time of day

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From the Department of Psychology and Neuroscience, CU-Boulder, Boulder, CO.

Serum glucocorticoid-dependent kinase 1 (Sgk1) regulates several types of ion channels, and thus may influence various functions in the brain. Sgk1 is also highly reactive to circulating glucocorticoids, the levels of which rise in the blood in a diurnal rhythm as well as in response to stress. Of the few studies characterizing expression of sgk1 mRNA expression in the brain, high expression was found in the CA3 region of the hippocampus, as well as upregulation of sgk1 mRNA in oligodendrocytes of the corpus callosum after acute stress or corticosterone (CORT) treatment. However, previous studies have not explored whether sgk1 mRNA shows a diurnal rhythm in the brain in response to diurnal CORT rise, nor whether expression during exposure to different circulating CORT levels are similar between white matter and neuronal regions. We characterized the expression of sgk1 mRNA in the brain of adrenal-intact rats (sham) or adrenalectomized (ADX) rats with or without 30 min acute restraint stress at two different zeitgeber times (ZT4 and ZT16). Sgk1 mRNA (in situ hybridization) was analyzed within the corpus callosum, hippocampus and two regions of the somatosensory cortex. In the corpus callosum, sgk1 mRNA was highly upregulated by elevations in circulating CORT. We
observed a robust time of day difference and an acute stress effect on sgk1 mRNA expression in sham rats, while ADX ablated both effects. In contrast, in hippocampus and cortex, the relationship between circulating CORT and the expression of sgk1 mRNA was more complex. The absence of CORT in those regions allowed a ZT difference in the expression of sgk1 mRNA that was not evident in sham rats and opposite to a ZT difference seen in acutely stressed adrenal-intact rats. Thus it appears that circulating CORT has different effects on sgk1 expression in neuronal regions compared to white matter. These results reveal strong and rapid modulation of gene expression in oligodendrocytes in response to acute stress and time of day. This observation may be especially noteworthy because both oligodendrocyte dysfunction and circadian dysregulation have been associated with psychological disorders.

**EXPERTISE:** In situ hybridization, qPCR, tissue culture, hormone assays, cloning, cell culture

58. Localization and physiological characterization of adipokinetic hormone (AKH) in the sea hare, *Aplysia californica*

**JOSHUA I JOHNSON, SI Kavanaugh, PS Tsai**

From the Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO.

Recent analyses have grouped gonadotropin-releasing hormone (GnRH), adipokinetic hormone (AKH), and corazonin (CRZ) into a superfamily of related peptides. Our lab has recently shown that *Aplysia californica*, an opisthobranch mollusk, is the first known protostome to simultaneously produce AKH (ap-AKH) and GnRH (ap-GnRH). As such, *A. californica* represents an excellent model for examining the functional divergence of two related peptides within a single species. As an initial step towards this goal, the present study examined the localization of ap-AKH transcript and peptide in the central nervous system (CNS) of *A. californica* using in situ hybridization (ISH) and immunocytochemistry (ICC), respectively. Additionally, in-vivo effects of ap-AKH were examined by a series of injection experiments. ISH studies detected ap-AKH transcript in neuronal cell bodies of the abdominal, pedal, and cerebral ganglia, as well as in some axonal processes of the cerebral ganglia, a result consistent with our previous RT-PCR data. ICC using a specific ap-AKH antibody was conducted on sections immediately adjacent to those used for ISH. Robust immunostaining for ap-AKH peptide was detected in the same neurons that also produced the transcript. Lastly, in-vivo injections of ap-AKH induced rapid weight loss, acutely inhibited feeding, and reduced ovotestis mass without affecting circulating glucose or hepatopancreas glycogen stores. Overall, our data indicate that ap-AKH is a biologically active neuropeptide produced predominantly in three central ganglia. Compared to our previous data on ap-GnRH, ap-AKH exhibits distinct patterns of expression and possibly target sites. Our data add to the growing body of literature on the functional evolution of the GnRH-related peptide superfamily. Further, they suggest these paralogous genes have undergone substantial neofunctionalization over the course of metazoan evolution.

**EXPERTISE:** Immunocytochemistry, in-situ hybridization, various carbohydrate assays, Aplysia physiology

59. ICV infusion of (pro)renin receptor antagonist (mPRO20) attenuates prorenin and DOCA-salt induced hypertension

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We previously reported that the binding of prorenin to (pro)renin receptor (PRR) plays a major role in brain Ang II formation and development of deoxycorticosterone acetate (DOCA)-salt hypertension. In this study, we designed and developed an antagonistic peptide (mPRO20) to block the binding of prorenin to PRR. Fluorescence-labeled mPRO20 bound to both mouse and human brain tissue, and the dissociation constant was 14.5nM and 0.9nM respectively. The binding was blocked by co-incubation with prorenin, indicating the specificity of mPRO20 to PRR. To test the in vivo effect of mPRO20, C57BL/6 mice were implanted with telemetric probes and intracerebroventricular (ICV) cannula. ICV infusion of mouse prorenin (300ng in 3µl for 10 minutes) increased BP ($\Delta$MAP: 28±3.4 mmHg); and this effect was attenuated by mPRO20 in a dose-dependent manner (maximum effect, $\Delta$MAP: 7±2.9 mmHg). Chronic ICV infusion of mPRO20 (40µg/kg/day, 21 days) attenuated the development of hypertension (113±3.3 vs. 134±3.6 mmHg, P<0.05), and the increase in brain Ang II levels (904±47 vs. 1371±88 pg/g) induced by DOCA-salt. In addition, ICV infusion of mPRO20 improved autonomic function and spontaneous baroreflex sensitivity in mice treated with DOCA-salt. In summary, mPRO20 binds to both mouse and human PRR, decreases Ang II formation and hypertension induced by either prorenin or DOCA-salt. We conclude that mPRO20 may be a novel PRR antagonist for the treatment of hypertension.

**EXPERTISE:** Telemetry recording, immunohistochemistry, autonomic function measurement.
60. A New Transgenic Mouse Model Reveals that Estradiol Regulates Ovine GnRH Receptor Expression through a Cyclic AMP Response Element in the Proximal Promoter.

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Estradiol (E2) regulation of the Gonadotropin Releasing Hormone Receptor (GnRHR) is observed in women and most domestic animals. To assess the contribution of the cyclic AMP response element (CRE) located in the proximal promoter of the ovine GnRHR gene, 9100 bases of the proximal promoter that had previously been fused to luciferase and used to construct transgenic mice (-9100oGnRHR-Luc), was mutated (µCRE) and used to construct three new lines (A-C) of transgenic (-9100µCREoGnRHR-Luc) animals. Offspring were assessed for luciferase (Luc) expression in the pituitary gland and other tissues. ANOVA (GLM) for each of the tissues was performed to evaluate Luc expression in transgenic vs. littermate non-transgenic animals. All transgenic females had higher (P<0.04) pituitary Luc expression than the transgenic males (P<0.05), which was higher than the non-transgenic animals (P<0.05). To elucidate the contribution of the CRE binding domain to E2 induced oGnRHR regulation, -9100oGnRHR-Luc and -9100µCREoGnRHR-Luc lines A and B transgenic females were ovariectomized and treated with E2 (2.5 mg pellet, SQ, Innovative Research of America) and/or GnRH antiserum (AS, 300 µL IP) using a 2x2 factorial design with F-tests to assess variance between treatments within transgenic lines. For all lines, AS alone decreased pituitary Luc expression (P<0.001) and there was no observed effect of E2 on pituitary Luc expression without concurrent AS treatment (P=0.4). In the -9100oGnRHR-Luc females, the combination of E2 and AS vs. AS alone increased (P=0.0003) pituitary Luc expression, while the mutated lines had no significant change in pituitary Luc expression in response to E2. These data suggest a critical role for a conserved proximal promoter CRE in mediated E2 regulation of oGnRHR expression. This work was supported by NIH R01 HD065943 “Physiological Mechanisms Underlying Heightened Responsiveness of Gonadotropes to GnRH”

61. Altered serotonergic gene expression associated with anxiety-like behavior after Crh receptor priming in the bed nucleus of the stria terminalis

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The bed nucleus of the stria terminalis (BNST) expresses the anxiety- and stress-related neuropeptide corticotropin-releasing hormone (Crh) and is a critical component in the neural systems controlling anxiety-related behavior. Recently, overexpression of Crh in the BNST has been shown to alter Crh receptor expression specifically in the anxiety-related serotonergic dorsal raphe nucleus, dorsal part (DRD). Here, we thus tested the hypothesis that repeated intra-BNST activation of Crh receptors induces a chronic anxiety-like state, and alters the expression of tph2 (the gene encoding the rate limiting enzyme for brain serotonin synthesis, tryptophan hydroxylase 2) and slc6a4 (the gene encoding the serotonin transporter, SERT) in the DRD or other midbrain serotonergic systems, using in situ hybridization histochemistry. Adult male rats were primed for five consecutive days with bilateral intra-BNST injections of vehicle (1% bovine serum albumin in 0.9% saline, n=11) or behaviorally subthreshold doses of urocortin 1 (Ucn1, n=11), a Crh-like peptide. Priming with Ucn1 elevated tph2 and slc6a4 mRNA expression selectively within the DRD, and increased anxiety-like behavior in the social interaction (SI) test. There was a strong negative correlation between tph2 mRNA expression in the DRD and SI time. These data, together with previous studies, are consistent with the hypothesis that Crh control of a BNST/DRD serotonergic system plays a key role in development of a chronic anxiety-like state. This system may play an important role in anxiety disorders as well as stress-induced relapse to drug taking.


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Abnormal sexual maturation and infertility in mice and humans result from disruption of Gonadotropin releasing hormone (GnRH) neuron development or function. Microarray analysis of GT1-7 (differentiated) as compared to NLT (undifferentiated) mouse GnRH neuronal cell-lines revealed an up-regulation of most Class II HDACs,
particularly HDAC9 (IIa, 6.7-fold) and HDAC6(IIb, 3.5-fold). Putative role of HDAC9 and 6 in GnRH biology was defined with a higher mRNA and protein levels and higher HDAC activity in GT1-7 cells, along with diminished ability of basal migration and pro-survival role in NLT cells. Since HDAC6 is exclusively cytoplasmic localized, we asked if the functional role of the domains and nuclear versus cytoplasmic location to mediate HDAC9’s pro-survival and migratory effects. Immunochemistry demonstrated WT HDAC9 was expressed preferentially in the nucleus (N>C), whereas HDAC-N was only nuclear and HDAC9-C (deacetylase domain) was exclusively cytoplasmic. In response to growth factor withdrawal induced apoptosis, both N and C-terminal (0.2-fold) HDAC9 mutants showed decreased death similar to WT (0.3 fold, p=0.04), however both WT and C-terminal (0.5-fold, p=0.005), but not N-terminal (0.7-fold, p=NS) mutants showed decreased neuronal migration, suggesting that cytoplasmic localization may be critical for cell specific effect on migration but not on survival. These data support the role of HDAC9 to act as a stop signal in GnRH neurons via a unique cytoplasmic rather than nuclear site of action. We further identified HDAC6 as an interacting partner to HDAC9 using immunoprecipitation. Silencing of both HDAC6 and HDAC9 in GT1-7 cells further augmented neuronal migration (5-fold) compared to silencing HDAC6 (2.4-fold) and HDAC9 (1.9-fold) alone. Overexpression (NLT) and Silencing (GT1-7) studies support the hypothesis that HDAC9 and HDAC6 together act as a cytoplasmic stop signal in GnRH neuronal cell migration.

EXPERTISE: Immunocytochemistry, real time qPCR, tissue culture, immunoblotting

63. Similar clock gene mRNA rhythmic expression in both male and female rats in medial prefrontal cortex.

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From the Department of Psych and Neurosci, University of Colorado, Boulder, CO.
Cellular clock genes are important in establishing and coordinating circadian rhythmicity of daily functions including sleep and eating cycles, as well as various cognitive processes. Disruptions to rhythmic clock gene expression have been linked to some psychiatric disorders including major depression and bipolar disorder, which have higher prevalence in females than males. The master clock of the body, the suprachiasmatic nucleus(SCN), is responsible for maintaining the body’s daily circadian rhythms and entrainment of those rhythms with the environmental light:dark cycles. Glucocorticoid hormone levels change over the course of the day as a result of internal circadian control of basal corticosterone(CORT) secretion, as well as a dynamic response to external stressors. In rats, females exhibit a greater amplitude of diurnal CORT secretions and stress-induced CORT levels than do males. CORT binds to glucocorticoid receptors(GRs) found in most brain areas except for the SCN. The promoter region of one of the core clock genes, Per1, contains a glucocorticoid response element. Thus, Per1 mRNA expression in most brain regions, expect for the SCN, is susceptible to changes in CORT, and therefore may exhibit differential expression between females and males. We tested whether there is an oscillating molecular clock within extra-SCN brain regions by examining the rhythmicity of the core cellular clock genes, Per1, Per2, and Bmal mRNA in subregions of the prefrontal cortex(PFC) including the prelimbic(PL) and infralimbic(IL) in both male and female rats. Rats were maintained on a 12:12 h LD cycle and then sacrificed at 6 different time points throughout the day(ZT0,4,8,12,16,20). Using in situ hybridization we found rhythmic clock gene expression in the PL and IL of males and females. Per1 and Per2 mRNA in the PL and IL were in-phase with each other, with peak mRNA levels at ZT16, which is antiphasic to the peak Per1 and Per2 mRNA in the SCN. On the other hand, Bmal mRNA in the PL and IL peaked at ZT20. There was not a significant sex difference in the phase or amplitude of the clock gene mRNA examined in the PL and IL of the PFC. Future studies will examine the effect of acute stress on clock gene expression of female and male rats.

EXPERTISE: in situ hybridization

64. New Tool to Modulate Levels of Endogenous Testosterone in Adult Male Songbirds.

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The songbird brain contains a network of specialized structures dedicated to song learning, performance, and perception. In males, several sites in this “song system” are affected by levels of circulating testosterone (T), which can influence cells through activation of androgen receptors or estrogenic metabolites. Changes in the levels of circulating T can induce changes in the number of neurons present, the activity of individual cells, and the learning and performance of song behavior. A long-standing goal in our field has been to understand the mechanisms through which changes in neuroendocrine status are manifest as changes in the expression of learned song behavior, and here we provide a new tool to facilitate those investigations. Experimental
approaches are presently limited to methods that elevate T beyond physiological levels or invasive methods to eliminate gonadal sources of T. Here we show that subcutaneous implants of the GnRH agonist Suprelorin (tradename: Deslorelin) are a safe and effective tool to modulate levels of endogenous T between those extremes. Groups of adult male zebra finches (Taeniopygia guttata) were recorded to determine their pre-drug status, then they were implanted with 1 mg/kg or 5 mg/kg doses and tested over durations terminating at 1, 2, 4, or 8 weeks. Each week, we recorded each bird's blood plasma T. In the final test for each bird, we also recorded song behavior and gross morphology of brain, skeletal muscle, heart and testes. Initial results reveal that T is suppressed in a dose-dependent fashion, and song changes associated with high doses are similar to those reported for castrated zebra finches. There were no changes in gross morphology, indicating that behavioral changes were not simply due to deteriorated health. In ongoing analyses, we are looking into possible changes in the song systems of these birds. Future experiments will explore the degree to which removal of the implant may restore T to control levels. Suppression of T using Deslorelin will be useful in cases in which gonadectomy surgery is too invasive or otherwise undesirable.

65. Roles of signaling pathways in the development and maintenance of the GnRH neuronal system.

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Hypothalamic gonadotropin-releasing-hormone (GnRH) neurons are critical for proper function and regulation of the hypothalamic-pituitary-gonadal (HPG) axis. Kisspeptin (KISS1) binds to its receptor, GPR54, located on the surface of GnRH neurons to stimulate GnRH peptide secretion. In this way KISS1 and GPR54 are established regulators of the HPG axis, and dysfunction of either has been implicated in hypogonadotropic hypogonadism exemplifying the potential importance of KISS1 signaling. The current study used immunohistochemistry to assess potential roles of GPR54-coupled Gq/11 and β-arrestin pathways in the development and maintenance of the murine GnRH neuronal system. Embryonic day 18.5 (E18.5) mice lacking functional β-arrestin1 and -2 had significantly fewer immunoreactive Gq/11 and β-arrestin pathways in the hypothalamic-pituitary-gonadal (HPG) axis. Kisspeptin (KISS1) binds to its receptor, GPR54, located on the surface of GnRH neurons to stimulate GnRH peptide secretion. In this way KISS1 and GPR54 are established regulators of the HPG axis, and dysfunction of either has been implicated in hypogonadotropic hypogonadism exemplifying the potential importance of KISS1 signaling. The current study used immunohistochemistry to assess potential roles of GPR54-coupled Gq/11 and β-arrestin pathways in the development and maintenance of the murine GnRH neuronal system. Embryonic day 18.5 (E18.5) mice lacking functional β-arrestin1 and -2 had significantly fewer immunoreactive GnRH neurons compared to wild type and heterozygous littermates. In addition, the effect of β-arrestin1 and -2 disruption is region specific. Adult mice lacking function β-arrestin1 had no differences in GnRH immunoreactive neuron count or distribution while GnRH immunoreactive neurons in mice lacking β-arrestin2 appeared to be impacted in a region specific manner. Finally, disruption of Gq/α11 appeared to affect the total number of GnRH immunoreactive neurons. These findings suggest differences in the functional importance of Gq/α11 β-arrestin1 and -2 in the development and maintenance of the GnRH neuronal system.

EXPERTISE: Cell culture, Immunohistochemistry, PCR, data analysis

66. Functional divergence of two evolutionarily related peptides in the sea hare Aplysia californica: in vivo comparison of gonadotropin-releasing hormone (ap-GnRH) and adipokinetic hormone (ap-AKH).

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Gonadotropin-releasing hormone (GnRH) and adipokinetic hormone (AKH) are grouped into the GnRH superfamily. Aplysia californica, a gastropod mollusk, is the first protostome shown to simultaneously synthesize an AKH (ap-AKH) and a GnRH (ap-GnRH) ortholog. Therefore, A. californica is an excellent model for examining the functional divergence of two related peptides within a single species. Previous localization studies in A. californica have shown that ap-AKH and ap-GnRH display distinct central nervous system (CNS) expression patterns, but it was unclear if these two peptides also played distinct physiological roles. The goal of the present study is to examine if ap-AKH overlaps with ap-GnRH in physiological effects previously described for ap-GnRH, such as parapodial opening and feeding inhibition. Further, we characterize novel biological effects unique to ap-AKH. To achieve these goals, we injected wild-caught A. californica with vehicle, ap-GnRH (15mg/animal), or ap-AKH (15mg/animal) and monitored physiological parameters including food consumption, body mass change, parapodial opening, and feces production. Within the timeframe examined, both food consumption and body mass were significantly reduced in animals injected with ap-AKH but not ap-GnRH. In contrast, parapodial opening was stimulated in animals injected with ap-GnRH but not
ap-AKH. Neither peptide significantly altered fecal production. Results from these studies show that ap-GnRH and ap-AKH produce reliable physiological responses upon injection, and that these responses are distinct from one another. These data, along with the unique CNS expression patterns of the peptides, support the idea that these two structurally related molecules have undergone substantial functional divergence over the course of metazoan evolution. Further, these data add to the growing body of knowledge on the functional evolution of members of the ancient GnRH family.

67. Conditional Deletion of Fibroblast Growth Factor Receptor 1 in GnRH Neurons Deleteriously Impacts Male and Female Reproductive Phenotype

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Fibroblast growth factor receptor 1 (Fgfr1) gene mutations are linked to hypogonadotropic hypogonadism in humans. Fgfr1 deficiencies in mice have led to several abnormalities associated with reproduction including a reduction in neurons that produce gonadotropin-releasing hormone (GnRH). Fgfr1 global deletion causes widespread disruptions in development. The goal of the present study is to use cre-lox strategy to produce transgenic mice with conditional Fgfr1 deletion in GnRH neurons. These transgenic mice will be used to investigate if Fgfr1 has a cell-autonomous effect on GnRH neurons. Mice were generated using male GnRH-Cre mice (GnRH-Cre+/-) breed with female mice with floxed Fgfr1 exon 4 allele (Fgfr1flox/flox). After two generations, male and female conditional knockout (KO) mice (GnRH-Cre+/-: Fgfr1flox/flox) and control mice (GnRH-Cre-/-: Fgfr1flox/flox) were produced. To investigate reproductive deficiencies in the conditional KO mice, the number of GnRH neurons was assessed along with reproductive measurements of pubertal onset, anogenital distance, and litter production. Body mass was also measured to check for developmental difference between genotypes. Results show no significant differences in GnRH neuron number between control and conditional KO at postnatal day 30 in either males or females. However, male conditional KO mice had significantly reduced body mass and anogenital distance, suggesting reduced androgen production. Female conditional KO mice exhibited significant delays in pubertal onset, age of first litter production, and decreased litter size, suggesting functional deficiencies within the female reproductive axis. These results suggest that conditional KO mice in both sexes have functionally compromised GnRH neurons despite normal GnRH neuronal populations.

68. Dietary N-methylserotonin 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 is a minor component of black cohosh that exhibits potent functional activity at the serotonin (5-HT) receptor isoform involved in thermoregulation (5-HT7) and is a weak serotonin reuptake transporter (SERT) inhibitor. This implicates NMS as an active component of black cohosh for hot flash and mood-related menopausal symptom relief, but the in vivo effects and effective dose(s) of NMS are currently unknown. In this study we sought to determine the effects of dietary supplementation of NMS on induced hot flash and mood in female rats. 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 in the open field, anxiety-like behaviors on the elevated plus maze, and depression-like behaviors with the forced swim test. Hot flashes were subsequently induced with intravenous (i.v.) calcitonin gene related peptide (CGRP) and skin temperature was monitored. Results indicated that NMS supplementation did not affect OVX-induced weight gain, uterine growth, or mood-related behaviors. However, NMS supplementation did significantly lower baseline skin temperature and blunted the hot flash response in a manner similar to estradiol implanted animals. These data argue that optimization of NMS content in black cohosh or NMS as a standalone supplement could provide relief from menopausal hot flashes without the risks associated with hormone replacement therapy or for patients concerned about phytoestrogens and their potential side effects.
69. The timing of Corticosterone peak circulation affects rhythmic clock gene expression in the rat prefrontal cortex.

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Extra-SCN tissues receive sparse direct neuronal connectivity from the hypothalamic suprachiasmatic nucleus (SCN); however glucocorticoid receptors (GR) are ubiquitously expressed in all areas of the body except for the SCN itself. This is an important distinction and suggests that CORT may be a key element in whole-body coordination of circadian rhythms. Although disrupted CORT circulation patterns have been shown to affect clock gene rhythmicity in certain peripheral tissues, virtually no characterization of this prospect has been examined in the brain. Studying the regulation of prefrontal cortex (PFC) circadian function in particular may be useful for understanding mechanisms of extra-SCN circadian entrainment in the brain. The PFC is a known modulator of emotional and endocrine control over the stress response. The PFC also expresses a high level of GR, but it receives no direct innervation from the SCN. It is possible that alteration of CORT secretion patterns could shift or disrupt core clock gene functioning in the PFC and result in dysregulation of its function.

To examine this possibility we have conducted experiments in which the presence and daily pattern of CORT exposure was manipulated in rats by adrenalectomy (ADX) ± daily CORT injection. In an initial experiment we examined per1 and bmal1 mRNA expression (in situ hybridization) in sham-ADX, ADX and ADX + daily CORT at ZT1 (CORT exposure pattern antiphasic to sham-ADX rats) treated male Sprague-Dawley rats. The absence of CORT did not abolish rhythmic expression of clock gene expression in the rat PFC. Surprisingly, antiphasic CORT treatment of ADX rats largely abolished rhythmic PFC clock gene expression, perhaps reflecting a disruption of the cooperative entraining influence of endogenous CORT and the SCN. Our manipulations had no effect on per1 and bmal1 expression in the SCN consistent with its lack of GR. In a follow-up experiment we have directly compared the effect of daily CORT treatment of ADX rats when CORT is injected at either ZT1 or ZT11 (maintained on 12:12 h LD). ADX rats were given an i.p. vehicle (n=16) or CORT (2.5 mg/kg, n=24) daily injection at either ZT1 or ZT11 for 13 days. On the 14th day rats were sacrificed at ZT0, ZT6, ZT12, and ZT18.

EXPERTISE: In situ hybridization, immunocytochemistry, rtPCR, ELISA, behavioral testing

Sensory and Motor Systems

70. The acoustical cues to sound location in the adult Guinea pig: measurements of Directional Transfer Functions (DTFs).

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There are three main acoustical cues to sound location, each of which is generated by the spatial-and frequency-dependent filtering of the propagating sound waves with the outer ears: Interaural differences in time (ITD) and level (ILD) as well as monaural spectral shape cues. The guinea pig has been a common model for studying the anatomy, physiology, and psychophysics of binaural and spatial hearing, yet little is known about their acoustical cues. Here, we measured the directional transfer functions (DTFs), the directional components of the head-related transfer functions, for 7 adult guinea pigs. DTFs were measured at both ears from 325 locations, with steps of 7.5º in both azimuth and elevation. The resultant localization cues were computed from the DTFs. In the frontal hemisphere, spectral notches were present for frequencies from ~13-20 kHz; in general, the frequency corresponding to the notch increased with increases in source elevation and in azimuth towards the ipsilateral ear. The mean maximum ITD observed across the guinea pigs was 251 ± 5.8 µs. In general, maximum ILDs were < 10 dB for frequencies < 4 kHz, and ranged from 10 dB - 30 dB for the frequencies from 4-24 kHz. Pinna removal eliminated the spectral notches and reduced both ILD and ITD cues. With respect to head and pinna size, the acoustical cues to sound location for the guinea pig are consistent with other mammals that have been studied. These results also emphasize the pinna’s role in generating these acoustical cues. Support: NIDCD R01-DC011555
71. The dependence of the binaural interaction component (BIC) of the auditory brainstem response (ABR) on binaural cues to sound source location in the guinea pig (Cavia porcellus).
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The sound-evoked BIC is the residual ABR after subtracting the binaurally-evoked ABR from the sum of monaurally-evoked ABRs. The β peak is the first negative peak of the BIC. Latencies of β in human and animal studies indicate a brainstem origin for this electrophysiological potential related specifically to binaural processing around the inferior colliculus. The inferior colliculus is a site of brainstem input convergence, of which many neurons are sensitive to binaural acoustical cues to location (e.g. interaural time (ITD) and level (ILD) differences). The BIC may be diagnostically important: altered β latencies and amplitudes in children and adults correlate with and predict long-term behavioral deficits in binaural processing associated with chronic conductive hearing loss. In humans, cats, chinchillas and guinea pigs, β amplitude depends systematically on binaural cues to location, exhibiting maximal amplitude for ITDs and ILDs of zero (midline sources); β is often no longer detectable once ITDs and/or ILDs exceed the physiological range. In this regard, the BIC is also informative for perception, as changes in β peak latencies and amplitudes occurring with varied stimulus ITD or ILD are correlated with psychophysical performance (lateralization, discrimination, binaural masking level differences) in normal and hearing-impaired subjects. In the guinea pig, BIC characteristics and its dependence on ITD and ILD are comparable to humans and other species.
EXPERTISE: auditory brainstem response (ABR)

72. Audibility Effects on Cortical Processing
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New findings suggest that hearing loss causes significant cross-modal and intra-modal cortical re-organization. However, it is not clear to what extent changes in cortical resource allocation associated with hearing loss are related to auditory deprivation versus decreased audibility of the signal. Examining the patterns of neurophysiological changes that occur in the brain close to hearing threshold in normal hearing individuals may help us to better understand the effects hearing loss on cortical functioning. The purpose of this study was to explore how cortical auditory evoked potentials (CAEPs) change with presentation level in two different auditory paradigms. The first experiment explored the effects of sensation level on CAEP latency, amplitude, and cortical activation patterns in normal hearing listeners. Responses were recorded using a 128-channel electrode net in response to a speech syllable stimulus at different presentation levels. The second experiment examined the effects of level on sensory gating in normal hearing subjects. Sensory gating is considered an index of the brain’s ability to filter out irrelevant stimuli and focus on information that is pertinent. Sensory gating was measured using an auditory paired click paradigm where a reduction in amplitude of the P1 and P2 components between the first and second click serves as a clinical biomarker of gating deficits. The results from both experiments provide insight into the dynamic changes that occur in auditory cortical processing close to threshold and elucidate recent findings from our laboratory demonstrating cortical reorganization as a result of auditory deprivation in early-stage hearing loss. Supported by NIH R01 DC0625.
EXPERTISE: High density EEG data collection, EEG source analysis and interpretation.

73. Spatial hearing capabilities of the adult guinea pig (Cavia porcellus).
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From the departments of Physiology and Biophysics, Otolaryngology, and the NTP at the University of Colorado AMC, Aurora, CO, and Neurobiology and Anatomy at the University of Rochester, Rochester, NY.
Despite common use of guinea pigs in investigations of the neural mechanisms of spatial hearing, their behavioral capabilities have not been investigated. We tested the spatial hearing of guinea pigs using pre-pulse inhibition (PPI) of the acoustic startle response. We conducted experiments aimed at quantifying 1) the minimum audible angle (MAA) and 2) the spatial release from masking (SRM); the physiological correlates which have been investigated in the guinea pig. Animals were placed in an acoustically transparent wire cage, with their head facing towards or 45° to the right of the midline of a 1m radius hemispheric ring of loudspeakers mounted every 7.5°, inside of a double walled sound attenuating chamber lined with acoustical foam. In the first experiment, continuous noise was presented from one speaker for at least 15s, then immediately swapped to another speaker a short time interval (5-300ms) before a loud (~110 dB SPL) startle eliciting stimulus was presented overhead. In all conditions, PPI was greatest for a 90° swap, and was systematically lower for
smaller angles. Thus, guinea pig MAA across the midline is at least 15° for broadband and low- and high-pass noise, and 30° for broadband noise offset by 45°. The second experiment was similar, except a variable intensity 200ms duration broadband chirp train (the pre-pulse in this condition) was played immediately before the startle eliciting noise burst, while a continuous broadband noise (~70dB SPL) was played from from a more lateral position (7.5-90° separation). PPI was weakest for chirps presented at a low intensity, from a nearby (7.5° separation) speaker, and increased with chirp intensity and angular separation. The results indicate that guinea pigs can: 1) discriminate changes in source location both within a hemifield as well as across the midline, 2) discriminate sources of low- and high-pass sounds, demonstrating that they can effectively utilize both low-frequency interaural time and high-frequency level difference sound localization cues, and 3) utilize differences in source locations to increase detection of a sound signal. Supported by NIDCD R01-DC011555, T32-DC012280.

**EXPERTISE:** Extracellular single cell recording, multi-barrel/site electrophysiological recordings and pharmacological manipulations, behavioral testing.

### 74. Comparative neuronal morphology of the cerebellar cortex in afrotherians, carnivores, cetartiodactyls, and primates

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Although the basic morphological characteristics of neurons in the cerebellar cortex have been documented in several species, virtually nothing is known about the quantitative morphological characteristics of these neurons across different taxa. To that end, the present study investigated cerebellar neuronal morphology among six different, large-brained mammalian species comprising a broad phylogenetic range: afrotherians (African elephant, Florida manatee), carnivores (Siberian tiger, clouded leopard), cetartiodactyls (humpback whale, giraffe) and primates (human, common chimpanzee). Specifically, several neuron types (e.g., stellate, basket, Lugaro, Golgi, and granule neurons; N = 317) of the cerebellar cortex were stained with a modified rapid Golgi technique and quantified on a computer-assisted microscopy system. Brain mass ranged from 78 g in the leopard to 4,990 g in the elephant. The cerebellar cortex in these species exhibited the trilaminar pattern common to all mammals. Morphologically, neuron types in the cerebellar cortex were generally consistent with those described in rodents (Palay and Chan-Palay, 1974), although there was substantial quantitative variation across species. In particular, Lugaro neurons in the elephant appeared to be disproportionately larger than those in other species. Furthermore, the data provided preliminary support for potential differences in dendritic measures for each species, but larger sample sizes are required.

**EXPERTISE:** Golgi staining

### 75. Super-resolution imaging of olfactory sensory neurons using a custom built STED microscope.

**BARIS N OZBAY**, SA Meyer, D Restrepo, EA Gibson.

From the Department of Bioengineering and the Department of Cell & Developmental Biology, University of Colorado Denver Health Sciences Center.

We built a two-color Stimulated Emission Depletion (STED) laser scanning microscope with the ability to resolve diffraction limited structures of neuronal dendritic processes. The microscope is designed similar to one developed by Johanna Bückers, et. al. (Opt. Exp. 2011) in the lab of Dr. Stefan Hell. The STED microscope images at Atto590/Atto647N wavelengths and is capable of doing so simultaneously. We characterized the resolution of the system by imaging 40nm fluorescent beads as ~58nm (Atto590) and ~44 nm (Atto647N). The microscope is part of the UC Denver Advanced Light Microscopy Core, primarily for use by neuroscientists. We used the STED microscope to image the clustering of transduction machinery in mouse olfactory sensory neuron cilia. Specifically, we were able to resolve sub-diffraction limited structures of the calcium permeable cyclic nucleotide gated (CNG) channel as well as the calcium sensitive chloride channel (ANO2). This suggests a complex organization of related transduction machinery, which may be important for the efficient
sensation of chemical signals. In the future, two-color STED may be used for investigating molecular-scale details of other neuronal signaling mechanisms, including synaptic processing.

EXPERTISE: Immunocytochemistry, time-dependent calcium imaging, loose patch electrophysiology

76. Cholinergic Neurotransmission Links Solitary Chemosensory Cells to the Immune System

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The nasal epithelium houses a population of Solitary Chemosensory Cells (SCCs) that respond to “bitter” substances and bacterial metabolites via the canonical taste transduction cascade (T2Rs, Gα-gustducin and TRPM5). SCCs release a hitherto unidentified neurotransmitter onto peptidergic (substance P+) trigeminal nerve endings to evoke protective respiratory reflexes. Until now the possibility that SCCs might also trigger neurogenic inflammation has gone unexplored. In the current study, we developed assays for measuring indicators of inflammation: edema, assayed as plasma leakage, and activation of the innate immune system, assayed as mast cell degranulation (MCD). To measure plasma extravasation (PE), we injected mice i.v. with Alexa555-conjugated albumin, then applied an irritant to the nose. To measure MCD, we stained the respiratory epithelium with acidified toluidine blue, which stains mast cell granules. In both experimental paradigms, mice were stimulated with Denatonium benzoate (Den, 10mM), which stimulates SCC, or with capsaicin (Cap, 2uM), which directly activates trigeminal nociceptors. Both Den and Cap elevated PE and MCD in normal mice, but only Caps did so in gustducin-/- or TRPM5-/- mice which inactivate SCC transduction. Thus for Den, SCC activation is required for induction of inflammation. Next, we tested mice whose TrpV1-expressing pain fibers had been ablated by resiniferatoxin. In those mice, both Den and Cap fail to evoke PE or MCD, indicating that trigeminal nociceptors are required for these effects. In the lower airways, brush cells, which are similar to SCCs in many respects, utilize acetylcholine (ACh) to signal to nerve fibers. To test whether SCCs too, which like brush cells express choline acetyltransferase, utilize ACh to activate pain fibers, prior to stimulation, we treated mice with the nicotinic ACh receptor (nAChR) blocker Mecamylamine (Mec). In mice treated with Mec, Den failed to induce PE or MCD, but Caps still evoked normal responses, indicating that nAChR's are required for SCCs to activate trigeminal fibers. In summary, we have outlined the pathway by which SCCs trigger neurogenic inflammation in the nasal epithelium first via ACh acting on nAChRs.

EXPERTISE: Immunofluorescence, confocal microscopy

77. Transgenic expression of endogenous calcium indicator GCaMP3 allows visualization of somatic and visceral sensory neurons in vivo.

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Somatic and visceral structures are innervated by many types of sensory fibers that are functionally distinct and highly plastic in response to injury and disease, but which properties and subpopulations of neurons are altered and to what extent is unknown. To adress this we crossed mice expressing GCaMP3 (an endogenous calcium indicator) downstream of a loxP-flanked CAG promoter with an EIIa-Cre line in order to induce GCaMP3 expression in all DRG neurons. When dissociated GCaMP3-expressing DRG neurons were examined in culture, low levels of GCaMP3 fluorescence were detected at rest. When stimulated with 50mM K+ to induce depolarization and allow Ca2+ influx, virtually every cell exhibited a Ca2+ signal with a αF twice that of Fura-2, but a longer decay constant (t1/2). Stimulation with K+ could be repeated (1/min for 20 mins) with no obvious decrease in signal. To determine if GCaMP3 could be used to report activity in somatic and visceral afferents in vivo, optical recordings were made in L6 and S1 in a decorticate preparation. Bipolar electrodes implanted in the base of the tail were driven with stimulus frequencies of 1, 10 and 100 Hz. A GCaMP3 signal was observed at all frequencies with increasing number of cells responding at higher frequencies, and the intensity of the GCaMP3 signal increased in individual cells with increased frequency of stimulation. GCaMP3 signals could also be elicited with brushing and radiant heat applied to the tail. Brushing of the tail produced strong signals in neurons with large diameter somata, while heating of the tail and perianal region elicited signals from neurons with small somata. Ramp distension (0-60 mmHg over 20 sec) and stepwise distension (at 20, 40, and 60 mmHg for 20 sec) of the bladder were able to produce GCaMP3 signals. DRG neurons responding to bladder distension exhibited both low (<40 mmHG) and high (>40mmHg) thresholds. Bladder afferents that responded throughout all distension pressures were also observed. These results demonstrate that genetically engineered GCaMP3-expression can be used for population studies of sensory neurons in vivo, allowing relatively high throughput analysis of new pain therapies.
Olfactory contrast enhancement is often proposed to arise through GABAergic inhibitory mechanisms that filter odor signals based on their strength. Here we examined a novel mechanism that could underlie such signal filtering within olfactory bulb glomeruli that depends on the commonly-observed ability of metabotropic glutamate receptors (mGluRs) to down-regulate GABA release. Because mGluRs are likely to be activated by extrasynaptic glutamate, their activation should occur when a glomerulus receives strong inputs from olfactory sensory neurons (OSNs); hence disinhibition and passage of excitatory signals should preferentially happen with strong inputs. Importantly, it has also been established that extrasynaptic glutamatergic signaling is robust in glomeruli. To test the role of mGluRs in glomerular signal processing, we performed patch-clamp recordings in rat olfactory bulb slices, first asking what effect mGluR activation has on inhibition from GABAergic periglomerular (PG) cells onto tufted cells, a critical regulator of activation of output mitral cells. We found that local puff-application of the Group II mGluR-specific agonist DCG-IV on a glomerulus profoundly reduced GABA release from PG cells onto tufted cells evoked by OSN stimulation, while also increasing the number of evoked action potentials in tufted cells. At the same time, an antagonist for Group II mGluRs (LY341495) enhanced GABA release from PG cells. Thus, activation of mGluRs in glomeruli can disinhibit tufted cells through modulation of GABA release, and these receptors can be activated by native glutamate transients. In terms of the mitral cell response, we found complex, biphasic effects of mGluR activation. Application of the agonist DCG-IV hyperpolarized mitral cells and reduced their spiking due to OSN stimulation; however, there was depolarization and enhancement in spiking upon immediate removal of DCG-IV. The enhanced excitation of mitral cells following the mGluR-mediated hyperpolarization could be due to hyperpolarization-activated cation channels. Our results suggest that mechanisms exist to support an mGluR-dependent glomerular signal filter, although the temporal dynamics of the filter could differ between the output tufted cells and mitral cells.

**EXPERTISE:** Electrophysiology