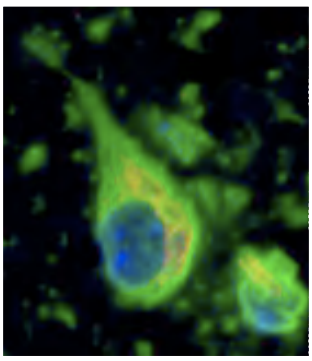
7th Annual Meeting of the

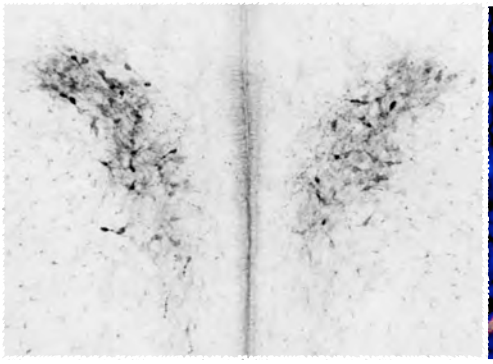
Front Range Neuroscience Group

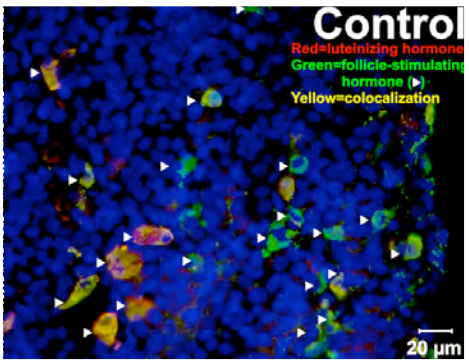
November 11, 2009

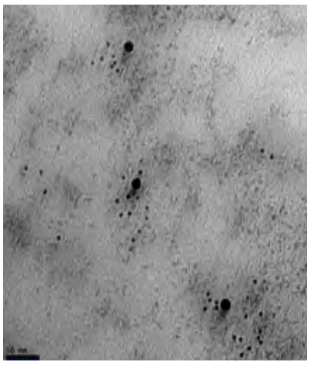
Marriott

Fort Collins, Colorado









Annual Meeting: November 11, 2009
Fort Collins Marriott
10:00am – 7:00pm

PROGRAM

10:30-11:15am - Workshop on Stereology
Dr. Won Yung Choi (Visiopharm Inc.)

11:15-noon - Data Blitz from multiple campuses

Noon-3pm – Lunch, Posters, Vendors!

3-4pm – Award Winning Student presentations
Heather Berens (UC Denver)
Arik Smith (Univ Wyoming)
Matt Stratton (Colorado State Univ)
Xihui Xu (Univ Wyoming)

4-5pm – Posters, Vendors, Coffee break

5-6pm –Keynote Lecture:
Dr. Catherine Woolley, PhD, Northwestern
Estrogen actions in the hippocampus:
It's all in the timing

6-7pm – Awards, door prizes, reception!

SfN http://FRNG.colostate.eduSfN

Institutional Sponsors 2009

Colorado State University

- Program in Molecular, Cellular and Integrative Neurosciences
- Department of Biomedical Sciences
- Program in Cell and Molecular Biology

University of Wyoming

- The Graduate School
- The Neuroscience Program

University of Colorado at Boulder

- College of Arts and Sciences, Dean's Office
- Howard Hughes Biological Sciences Initiative

Additional participating institutions:

- University of Colorado Denver School of Medicine
- University of Colorado Denver
- University of Denver
- University of Northern Colorado
- Colorado State University – Pueblo
- Regis University
- University of Nebraska-Kearney

BRAIN AWARENESS WEEK 2010!!!

Neuroscience Outreach Program at Ft Collins High Schools:
(Upcoming event: Fossil Ridge High School, 1-2 weeks in March)

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.

- **Share your knowledge about the nervous system, behavior and research**
- **Practice your skills in communicating science concepts and refine your teaching style**

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

Contact: Cynthia Smeraski, PhD (BAW Director), Department of Biomedical Sciences
Colorado State University,
email: cynthia.smeraski@colostate.edu
Phone: 970-491-8143

Corporate Sponsors 2009

PLATINUM:

ADInstruments, Inc.
2205 Executive Circle
Colorado Springs, CO 80906
Contact: Greg Boulet
Toll Free: 888-965-6040 x223
Tel: 719-459-6272
CO Phone: 719-576-3970 x223
Fax: 719-576-3971
Email:**gboulet@adinstruments.com**
Website:
http://www.adinstruments.com

Martek Biosciences Corporation
Christopher M. Butt, Ph.D.
Principal Scientist
Neuroscience Research, Discovery
4909 Nautilus Court North, Suite 208
Boulder, CO 80301
303.357.2841 (phone)
303.381.8181 (fax)
Email: **CButt@martek.com**
Website: **http://www.martek.com/**

MARTEK
life enriched.

North Central Instruments Inc. (Leica)
Formerly: E. Licht Company
5961 E. 38th Ave.
Denver, CO 80207
Contact Persons: Gary Hanson,
Scott Carter and Lance Russell
Phone: 303-322-8900
Email: **lancer@ncimicro.com**
scottc@ncimicro.com
Website:
http://www.northcentralinstruments.com/

GOLD:

Leeds Precision Instruments
800 Boone Avenue North
Minneapolis, MN 55427
Contact Person: Jeff Bright
Phone: 303-683-5524
Email: **jbright@leedsmicro.com**
Website: **http://www.leedsmicro.com**

Nikon Instruments, Inc.
3275 Denargo St. #930
Denver, CO 80216
Contact Person: Andrew Cahill
Voice: 303-217-0824
Email: **afcahill@Nikon.net**
Website: **http://www.nikon.com**

Fisher Scientific Company
4700 Moline St.
Denver, CO 80239
Contact Person: Anjanette Lambert
And David Holden
Customer Service: 800-766-7000
Phone: A Lambert: 303-596-5485
Email:
anjanette.lambert@thermofisher.com
david.holden@thermofisher.com
Website: **http://www.fishersci.com**

Stoelting Co.
620 Wheat Lane Wood
Dale, IL 60191
Contact: Richard Mills
Toll Free: 800-860-9775
Tel: 630-860-9700
Fax: 630-860-9775
Email: **richard@stoeltingco.com**
Website: **http://www.stoeltingco.com**

The Jackson Laboratory
610 Main Street
Bar Harbor, Maine 04609
Contact Person: Richard Longeras
Phone: (405) 255-0255
Email: **richard.longeras@jax.org**
Web: **http://jaxmice.jax.org/**

SILVER:

Promega US
2800 Woods Hollow Road
Madison, WI 53711
Contact Person: Jennifer Naylor, Ph.D.
Phone: 303-324-9746
Toll Free: 800-356-9526, ext. 2804
Email: **jennifer.naylor@promega.com**
Website: **http://www.promega.com**

Millipore Corporation
80 Ashby Road
Bedford, MA 01730-2271
Contact Person: Irv Crump, Candace Sotwick
Phone: IC 303-396-3580
Phone: CS 800-645-5476 x6870
Email: **irv_crump@millipore.com**
Email: **Candace_Sotwick@millipore.com**
Website: **http://www.millipore.com**

NextGen Pharma Technologies, Inc.
1235 Lake Plaza Drive, Suite 134
Colorado Springs, CO 80906
Contact Person: Cheryl Hite
Phone: 970-222-3844
Email: cheryl@nextgenpharma.com
Web: **http://www.nextgenpharma.com/**

LightLabs
PO Box 742513
Dallas, TX 75374
Contact Person: Matt Ver Meer
Phone: 800-286-3103 x704
Fax: 972-479-1910
Email**: Matt@lightlabsusa.com**
Website**: http://www.lightlabsusa.com**

Fermentas Inc
798 Cromwell Park Drive
Glen Burnie, MD 21061
Contact Person: Dan Golin
Cell Phone: 303-514-7584
Email: **Golin@Fermentas.com**
Voice: (800) 340-9026
Fax: (800) 472-8322
e-mail: **info@fermentas.com**
Website: **http://www.fermentas.com/**

Rainin Instrument, LLC
7500 Edgewater Drive
P.O. Box 2160
Oakland, CA 94621-0060
Contact person: Sarah Harris
Phone: 303-332-7288 Email:
Sarah.Harris@rainin.com
Website: **http://www.rainin.com/360_concept.asp**

Acknowledgements:

Cover Page: Designed by Karin Streifel
Scientific images provided by Heather Berens, Xihui Xu, Arik Smith and Matt Stratton. Details will be in their presentations in the oral session. The FRNG website (http://FRNG.colostate.edu) was created by Leif Saul in 2005 – see more images on our website: FRNG.colostate.edu. Thanks to Leif for creating our electronic abstract submission system for this year!!

Special Thanks!

Special thanks to faculty and postdoctoral fellows from all campuses for reading and judging abstracts and/or posters that provide the energy to create this meeting.

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

Special thanks to the vendors listed in this program, and to Cheryl Hite for helping coordinate their participation. 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: ADInstruments, Martek Biosciences Corporation, and North Central Instruments/Leica!!

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, and the University of Colorado at Boulder.

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 Cheryl Hartshorn, Karin Streifel, and Krystle Frahm from CSU, Dane Jensen and Andrew Young from Univ Wyoming, Tianna Hicklin, Jenn Whitesell, and Faye Doherty from UCHSC, Leah Brooks and Jessica Babb from UC-Boulder and George Talbott from DU. And additionally to Erin Bisenius and the first year CSU students Matthew, Susan, Elizabeth, Christina and Natalie for help with attendee registration.

Special thanks to the Marriott Fort Collins for providing the ideal venue and an extra contribution, and to Jamie Meyer in particular for help in making this all possible.

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,
Noreen Reist, Stuart Tobet, Shane Hentges, Qian-Quan Sun, Serge Campeau, Nancy Lorenzon, Mark Basham, Sondra Bland, and Patrick Burns.

THANK YOU TO ALL WHO DONATED RAFFLE PRIZES

Backcountry Provisions
140 N College Ave
Fort Collins, CO 80524
970.482.6913
www.backcountryprovisions.com

The Cupboard
152 S College Ave
Fort Collins, CO 80524
970.493.8585
800.569.1873
www.thecupboard.net

Kilwin's Chocolate and Ice Cream
114 S College Avenue
Fort Collins, CO 80524
970.221.9444
www.kilwins.com

Mad Greens
2120 E Harmony Rd
Fort Collins, CO 80528
970.223.1447
www.madgreens.com

Panera Bread Company
3700 S College Avenue
Fort Collins, CO
970.204.4500
www.panerabread.com

Paninos Restaurant
310 Prospect Rd
Fort Collins, CO 80526
970.498.8292
www.paninos.com

Rustic Oven
2350 E. Harmony Rd
Fort Collins, CO 80528
970.226.2300
www.rusticoven.com

Spicy Pickle
2120 E Harmony Rd
Fort Collins, CO 80528
970.377.2751
www.spicypickle.com

Two Pairs Nail Spa
115 E Mountain Ave
Fort Collins, CO 80524
970.482.2282
www.twopairsnailspa.com

Trail Head Tavern
148 W Mountain Ave
Fort Collins, CO 80524
970.221.5757

Walrus Ice Cream
125 W Mountain Ave
Fort Collins, CO 80524
970.482.5919
www.walrusicecream.com

WORKSHOP ON STEREOLOGY

Why stereology?: How unbiased quantification will benefit your research

Stereology in neuroscience provides practical techniques for extracting quantitative information about three dimensional structures in your brain sample based on measurements made on two-dimensional planar optical sections acquired from microscopic images. It is an unbiased method of estimating important features such as total cell count, volume, surface area, length, volume density and numeric density. When performed correctly, it makes no assumptions between your test groups, yields measures that are statistically correct, and is fast and efficient. In this workshop, an overview of the benefits of stereology will be discussed with examples from neuroscience using commonly used stereological methods.

The speaker:

Dr. Won Yung Choi is a Senior Products Scientist at Visiopharm's New York office. She also holds a joint appointment as an Adjunct Research Scientist at Columbia University Medical School. She is a neuroscientist with over 10 years of experience studying the dopaminergic system in various transgenic models and patient populations. She obtained her B.Sc. from McGill University in Montreal, Canada, and M.A., and Ph.D.in Neuroscience from Columbia University. Her dissertation focused on the functional dissociation of dopamine receptor subtypes and dopamine receiving regions in drug addiction related learning mechanism. She received her postdoctoral training at Columbia University and the New York Psychiatric Institute, focusing on the microanatomy and functional circuitry of the dopaminergic system using various genetic and molecular biological methods, advanced microscopy and stereology.

'DATA' BLITZ: Front Range Style

CU Boulder
Don Cooper: Institute for Behavioral Genetics with a focus on animal models of addiction.

Chris Lowry: Research programs in the Department of Integrative Physiology

Serge Campeau: Department of Psychology and Neuroscience's investigators focusing their research programs in the broad topic of stress neurobiology.

CU Denver Heath Science Center
Linda Barlow: Neural development

Mark Dell'Acqua: Synaptic transmission and plasticity and ion channel regulation with relevant links to epilepsy, Alzheimer's and Down syndrome

Dan Tolin: Systems neuroscience, which would include the groups studying taste, smell, and audition

University Denver/ Department Biological Sciences and Eleanor Roosevelt Institute
Scott Barbee: The molecular mechanisms underlying synaptic plasticity

Dan Linseman: Neurodegenerative diseases and cognitive disabilities

Nancy Lorenzon: Biophysics in the Departments of Biological Sciences, Chemistry & Biochemistry, and Physics & Astronomy

Colorado State University (Molecular, Cellular and Integrative Neuroscience Program)
Michael Tamkun: Potassium channel localization and function

Ronald Tjalkens: Neuroinflammatory mechanisms in Parkinson's Disease

Patricia Davies: Research involving systems and humans, including computational biology modeling, neuronal circuitry and systems, sensory biology and perception, behavior, neurophysiology,
neurorehabilitation, and development.

University of Wyoming
Andrew Young: Representing the Lab of Dr. Qian-Quan Sun. "Developing cortical circuits and their plasticity."

Andrew Taylor: Representing the lab of Dr. Donal Skinner. "Mechanisms driving seasonal prolactin secretion."

Jonathan Prather: Representing his own lab. "We know that adult behavior is shaped by juvenile experience, and we found within the adult songbird brain an explicit representation of juvenile experience."

Regis University
Mark Basham: Undergraduate Neuroscience Major with cell, molecular, genetic, and cognitive program components.

ORAL PRESENTATIONS

1. Requirement of Bax for neuronal apoptosis in viral encephalitis
Heather M. Berens and Kenneth L. Tyler.
Program in Neuroscience, University of Colorado Denver Anschutz Medical Campus

2. Chronic GnRH Agonist Exposure Selectively Decreases FSH-Immunoreactive Cells in the Male Rat Pituitary
A. Smith[1], C.S. Asa[2], W.J. Murdoch[3] and D.C. Skinner[1]
Neuroscience Program, University of Wyoming, Laramie, WY[1] Research Department, Saint Louis Zoo, Saint Louis, Missouri[2] Reproductive Biology Program, University of Wyoming, Laramie, WY[3]

3. GABA and the developing Paraventricular Nucleus of the Hypothalamus
Matt Stratton, Kristy McClellan, and Stuart Tobet
Department of Biomedical Science, Colorado State University, Fort Collins, CO

4. Neurokinin 3 receptor associates with active chromatin in the nuclei of paraventricular neurons following acute hyperosmotic challenge
Xihui Xu, Zhaojie Zhang, Dane Jensen, and Francis W. Flynn
Graduate Neuroscience Program, Department of Zoology and Physiology, University of Wyoming, Laramie WY

1. Requirement of Bax for neuronal apoptosis in viral encephalitis
Heather M. Berens and Kenneth L. Tyler.
Introduction: Reovirus infection of neurons is a major experimental model for understanding the pathogenesis of viral CNS infections. Like major human diseases including Herpes and West Nile encephalitis, reovirus induces apoptosis in CNS neurons. Both the extrinsic death receptor-mediated and the intrinsic mitochondrial pathways are involved in reovirus-induced apoptosis. Bcl-2 and its family members have vital roles in regulating the intrinsic pathway and release of pro-apoptotic factors from the mitochondria. Bax is the main pro-apoptotic executioner Bcl-2 family member in neurons. It resides in the cytoplasm of a healthy cell, and during apoptosis it translocates to the mitochondria to form pores in the outer membrane resulting in the release of pro-apoptotic factors into the cytoplasm. Due to the key role of Bax in neuronal apoptosis in models of growth factor withdrawal and ischemia, we wanted to investigate its role in reovirus-induced apoptosis by analysis of infected neonatal mice.
Methods: Two-day old NIH Swiss Webster mice or Bax-/- mice and their wild-type littermates were inoculated intracerebrally with Type 3 human reovirus. Mice were sacrificed when clinical disease was evident, and brains were collected for histological and immunofluorescence examination for Bax activation. Bax-/- mice were followed for signs of clinical disease and survival. Brains were harvested when mice were moribund, or at times when wild-type mice were moribund for comparison. Brains were processed for histopathology or viral titer and caspase activation.
Results: Bax is activated in infected neurons of the cortex, hippocampus, and thalamus and not in control mock infected mice. These areas are the classic sites of reovirus infection of neurons and CNS injury. In comparison to wild-type littermates, Bax-/- mice show delayed signs of disease, significant delay of death ($P < 0.001$), and an increase in survival (19% vs 0%). Bax-/- mice have decreased injury by histopathology in brain regions infected by reovirus. At day 9 post-infection viral titers are significantly decreased in Bax-/- mice ($P = 0.002$), and the executioner caspase 3 is not activated in Bax-/- brains while it is in wild-type mice.
Conclusions: The results indicate that Bax becomes activated in infected neurons in key areas of reovirus-infection and subsequent apoptosis. Bax may be important for viral growth within the CNS, and is important for apoptotic signaling during viral encephalitis. These studies suggest that inhibiting Bax activation, mitochondrial signaling pathways, or apoptosis more generally may provide novel therapeutic strategies to reduce CNS injury due to viral infection.

2. Chronic GnRH Agonist Exposure Selectively Decreases FSH-Immunoreactive Cells in the Male Rat Pituitary

A. Smith[1], C.S. Asa[2], W.J. Murdoch[3] and D.C. Skinner[1]
Neuroscience Program, University of Wyoming, Laramie, WY[1] Research Department, Saint Louis Zoo, Saint Louis, Missouri[2] Reproductive Biology Program, University of Wyoming, Laramie, WY[3]

Gonadotropin-releasing hormone (GnRH) agonists have emerged as an important animal contraceptive and a common treatment for several human disorders including advanced prostate cancer, endometriosis, uterine fibroids, and central precocious puberty. GnRH agonists work largely by inhibiting the secretion of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from gonadotropes in the anterior pituitary gland. It is known that constant exposure to GnRH desensitizes GnRH receptors. However, this desensitization effect fails to explain the sustained, although usually not permanent, suppression of the reproductive axis after cessation of treatment. Surprisingly, the effect of chronic GnRH exposure on pituitary cytology has not been determined. Here, we investigated the relationship between pituitary immunoreactive gonadotropes and serum gonadotropins following treatment with the GnRH agonist deslorelin and determined the effect of testosterone on these parameters.

Male Sprague-Dawley rats (135.9±0.2 days old) were treated with 1.1mg deslorelin implant only, deslorelin + sc testosterone; sc testosterone only; or sham implant insertion. After 6 weeks, rats were killed and, following plasma collection, transcardially perfused with paraformaldehyde. The pituitary, testes, and epididymi were weighed. Using standard immunocytochemical techniques, mid-sagittal pituitary sections were fluorescently labeled for LHβ and FSHβ. The number of single and dual-labeled cells and the total number of cells were determined. Plasma FSH and testosterone were estimated by EIA. Deslorelin with or without testosterone replacement significantly reduced the weight of the testes and epididymi compared to control animals. Further, deslorelin-treated animals showed significantly suppressed plasma FSH and testosterone levels compared to controls. There were three-fold fewer FSH-immunoreactive cells in deslorelin-treated animals, and this effect was not attenuated by testosterone replacement. Interestingly, LH-immunoreactive cells were not significantly affected by any treatment. These seminal data strongly suggest that GnRH agonists selectively affect FSH-immunoreactive cells in the rat pituitary. As FSH plays a critical role in spermatogenesis, our data may explain, at least in part, the delayed, or complete failure to, return to full spermatogenesis after discontinuing treatment with GnRH agonists that has been noted in some species. In ongoing studies, we are using orchidectomized rats to determine the possible role of non-androgenic testicular factors in the observed effect of deslorelin on pituitary gonadotropes.

3. GABA and the developing Paraventricular Nucleus of the Hypothalamus

Matt Stratton, Kristy McClellan, and Stuart Tobet
Department of Biomedical Science, Colorado State University, Fort Collins, CO

The paraventricular nucleus (PVN) of the hypothalamus is a central player in the regulation of many physiologic functions that include food intake, stress responses and cardiovascular function. It is the master controller of the hypothalamic-pituitary-adrenal (HPA) axis. Major depressive disorder (MDD) has been associated with a dysregulation of HPA axis function. Interestingly, there is evidence for increased likelihood of major depressive disorder in individuals with mutations in genes encoding GABAB receptors. Gamma-aminobutyric acid (GABA) is a neurotransmitter that during development acts as a secreted factor that regulates neuronal migration, proliferation, and differentiation. We hypothesize that some of the increased predisposition to MDD may be mediated by altered formation of the PVN during development due to alterations in GABA signaling. To test this hypothesis we are investigating the role of GABA in fetal PVN development in transgenic and knockout mouse models. Mice lacking the R1 subunit of the GABAB receptor at embryonic day 17 (E17) have altered distribution of cell containing immunoreactive estrogen receptor-alpha in and around the PVN as well as decreased immunoreactive BDNF within the PVN. We have also identified a decrease in the number of neurons containing immunoreactive

estrogen receptor-alpha in and around the PVN in response to fetal exposure to the GABAA receptor antagonist bicuculline (also at E17). Additional preliminary data from in vitro video microscopy indicates altered movement characteristics of cells in the developing PVN in response to the GABAB receptor antagonist saclofen. We have also demonstrated the feasibility of labeling fetal mitotic events in vivo and in vitro by incorporation and detection of 5-ethynyl-2'-deoxyuridine (EdU). By examining the location and proliferation of cells characteristic to the PVN, along with live cellular responses both by video microscopy and pharmacological indices of signaling we are determining key factors responsible for the formation of the PVN early in development.

4. Neurokinin 3 receptor associates with active chromatin in the nuclei of paraventricular neurons following acute hyperosmotic challenge

Xihui Xu, Zhaojie Zhang, Dane Jensen, and Francis W. Flynn
Graduate Neuroscience Program, Department of Zoology and Physiology, University of Wyoming, Laramie WY

Tachykinin neurokinin 3 receptors (NK3R) are membrane-bound G-protein-coupled receptors (GPCR), expressed by approximately 72% of magnocellular vasopressin (VP) neurons in the paraventricular nucleus (PVN) of the hypothalamus. Hypersmotic challenge (intra-gastric load of 6.0ml 2.0M NaCl) causes the synaptic activation of NK3R; the NK3R is internalized, and VP is released into the circulation. Immuno-electron microscopy (EM) showed that following osmotic challenge NK3Rs were translocated to the nucleus of PVN neurons where NK3R immuno-gold labeling appeared in punctuate groupings. The nucleus is a heterogeneous structure that is comprised of both condensed and decondensed chromatin and the distribution of immunogold NK3R suggested an association with chromatin. Chromatin is comprised of histones (H2A, H2B, H3 and H4) around which eukaryotic DNA is packaged. Histones are subject to many types of post-translational modifications and acetylation of H3 and H4 is generally recognized as a marker of gene activation. The experiment used double (NK3R and H4) immuno-TEM labeling to test the hypothesis that upon translocation to the nucleus, NK3R associates with chromatin. Rats were administered an acute hyperosmotic challenge (2 M NaCl) or no challenge. Rats were sacrificed 40 min later, the PVN isolated, and the nuclear fraction collected. Ultrathin sections of PVN nuclei were incubated in antibodies against NK3R, and H4 or acetylated H4, and then with the specific secondary antibodies conjugated to 15nm (for NK3R) and 6nm gold particles (for histones). Based on previous research, we defined a co-localization of NK3R and H4 as the gold bead reporters being within 60nm. Results from the double-immuo TEM indicated that NK3R labeling density is 0.8 beads/μm2 in the nuclei from control rats. The nuclear NK3R density increased to 18.5 beads/ μm2 in PVN nuclei isolated from rats administered 2 M NaCl. Within these nuclei from the hyperosmotic challenged rats, there are about 55% of NK3Rs forming clusters of 2-3 NK3R immunogold beads. Interestingly, the dimerization of nuclear NK3R matches the weight of NK3R detected by western analysis. Less than 10% of NK3R was spatially associated with H4 in control nuclei but following hyperosmotic challenge, the vast majority(95%) of NK3R co-localized with Histone H4. While these data show that NK3R co-localizes with H4 the antibody did not distinguish between the different posttranslational modifications of the H4, which would indicate if H4 is associated with silenced or active chromatin. Therefore the experiment was repeated using an antibody against acetylated H4. In control nuclei NK3R rarely co-localized with acetylated H4. However, following acute hyperosmotic challenge, there are about 60% NK3R co-localized with acetylated H4. The results establish in vivo for the first time that the membrane-bound NK3R associates with transcriptionally active regions of chromatin after hyperosmotic challenge. The translocation of the NK3R to nuclei of brain cells, as well as other cell types, provides additional signaling diversity. Supported by NIH grants: RO1 DK50586, RO1 NS 57823 and P20 RR15640 to F.W.F.

POSTER PRESENTATIONS

Cognition and Behavior

1. Effects of neonatal exposure to progesterone on the development and expression of adult male sexual behavior in the rat. AB Breton, MG Leedy, KJ Austin, and BM Alexander.

2. CSF Aβ40 and Aβ42 levels and cognitive dysfunction in the senior beagle.
Christina de Rivera, Joseph A. Araujo, Bill Milgram.

3. Perturbation of Molecular Pathways underlying behavior.
M Ahemed, D Dubach, X Huang-Sturgeon, A Costa, K. Cios, C Nguyen and K.Gardiner

4. Shared neural circuitry for selection and initiation of actions. Gidon Felsen and Zachary F. Mainen.

5. Evidence-Based Practice for Early Cognitive Impairment Detection on Older Individuals with Metabolic Syndrome. PC Heyn and Onofrei L.

6. STEP mediates ethanol inhibition of NMDARs, long-term potentiation and fear conditioning. TR Hicklin, Wu PH, Radcliff RA, Freund RK, Goebel-Goody SM, Proctor WR, Lombroso PJ, Browning MD.

7. An economical quantitative ratiometric method for detecting cocaine and benzoylecgonine for forensic and biological applications. DC Cooper, B Cadle, KC Rasmus, A Hannum, MM Marinelli, LS Leverich.

Development

8. Characterization of the oxytocin system in fibroblast growth factor 8 hypomorphic mice. LR Brooks, WCJ Chung and PS Tsai.

9. Heart field reduction in embryos depleted for voltage-gated calcium channel b subunit CACNB2. Yelena Chernyavskaya, Alicia Ebert, and Deborah Garrity.

10. Cell survival and proliferation are disrupted in the olfactory placode of fibroblast growth factor 8 hypomorphic mouse embryos. WCJ Chung and P.S. Tsai.

11. Reliability of ERN in Children Performing a Visual Flanker Task. PL Davies and WJ Gavin.

12. Reliability of ERN in Adults Performing a Visual Flanker Task. WJ Gavin and PL Davies.

13. Calcium channel MAGUK gene CACNB4 required for mitosis in zebrafish early development. Cory J. Harrell, Alicia M. Ebert, William B. Horne, Deborah M. Garrity.

14. Developmental Profile and Sexually Dimorphic Expression of Kisspeptin in the Mouse Brain. JG Knoll, CM Clay, GJ Bouma, TR Henion, GA Schwarting, RP Millar, SA Tobet.

15. Role of nitric oxide in the expression of gonadotrophin releasing and gonadotrophin inhibiting (RFamide related peptide) hormone in mouse development. Pankaj Kumar, Kazuyoshi Tsutsui, Paul Huang, Stuart Tobet.

16. Enriched environments protect against depression brought about by chronic mild stress and increase neuronal density in the dentate gyrus in Sprague-Dawley rats. McKenzie Letendre, ME Basham.

17. Determining the role of the Tbx5 transcription factor in zebrafish cardiac development. LE Parrie, YA Chernyavskaya, DM Garrity.

Disorders of the Nervous System

18. Overexpression of amyloid precursor protein induces mitochondrial oxidative stress and intrinsic apoptosis cascade. Bartley, MG, Linseman, DA.

19. Polymeric Nanowire Templates as Scaffolds for Improved Neuronal Cell Functionality. SL Bechara, KC Popat.

20. Neuroadaptations in mesolimbic dopamine neurons mediate avoidance behavior to social defeat and antidepressant actions. JL Cao, M Wilkinson, HE Covington III , EJ Nestler, DC Cooper and MH Han.

21. Characterization of the AdeI Mutant of Chinese Hamster Ovary Cells: A Cellular Model of Adenylosuccinate Lyase Deficiency. N Duval, LK Vliet, TG Wilkinson II, GN Vacano, D Patterson.

22. Selectivity of the protective effects of docosahexaenoic acid (DHA) in the N2A neuronal cell line. ML Florez-McClure, AE Garrison, and CM Butt.

23. RNA interference against hypoxia-upregulated 1 (Hyou1). DL Jost, A Poczobutt, S Jones, K Jonscher, D Leake, C Yamada, L Barrows, M Das, WM Zawada.

24. Neuroprotective effects of anthocyanins on mitochondrial oxidative stress induced neuronal death. NA Kelsey, WA Hulick, and DA Linseman.

25. Iron Accumulation in Mouse Huntington's Disease Brain Occurs in Neurons.
E. Marks, K. Dorsey, Steven Hersch, Barry Lai, Jonathan Fox.

26. Cardiovascular and functional benefits of acupressure following stroke. KL McFadden, S Huerta, TD Hernández.

27. Characterization of cerebellar Purkinje cells harboring a malignant hyperthermia mutation (Y522S) in the intracellular Ca2+ release channel, RyR1. Jason A. Santiago, George C. Talbott, Nancy M. Lorenzon.

28. Reovirus myelitis and glial activation. SA Schittone and KL Tyler.

29. Signal transducer and activator of transcription-5 plays a novel role in neuronal apoptosis induced by inhibition of Rac GTPase. TR Stankiewicz, FA Loucks, EK Schroeder, RJ Bouchard, DA Linseman.

30. Regulation of MCT1 Function By Intracellular pH and PKA Agonists in Cerebrovascular Endothelial Cells. AL Uhernik, C Tucker, JP Smith.

31. A novel role for Bcl-2 in the regulation of mitochondrial glutathione transport.
HM Wilkins, J Schwarz, and DA Linseman.

Neural Excitability, Synapse and Glia

32. Microglial response in the nucleus of the solitary tract after taste nerve damage. DL Bartel, TE Finger.

33. Knockout of TRPC5 channels in adult prefrontal cortex reduces burst-triggered depolarization and enhances cocaine reward. DC Cooper, MA Fowler, M Hu, L Li, A Dietrich, L Birnbaumer.

34. BDNF dependent synaptic plasticity underlies some forms of long-term memory. GP Cortese, TR Chapman, RM Barrientos, SF Maier, and SL Patterson.

35. Expression of importin ß-1 in the rat hypothalamus and its involvement in the nuclear transport of the membrane, neurokinin 3 receptor. DD Jensen, SM Lyden and FW Flynn.

36. Dopamine D1 receptor activation induces synaptic plasticity via a novel NR2B-dependent mechanism and promotes memory consolidation. LS Leverich, CE. Lane, JA Varela, RW Green and DC Cooper.

37. The avian ciliary ganglion: a useful model for the study of beta amyloid effects on neurotransmitter release. Susan Mazalan, Elizabeth Hart, and D. Bruce Gray.

38. Sub-Pathologic doses of MPTP stimulate glial activation in nuclear factor kappa B (NF-kB) reporter mice. JA Miller, DL Carbone, JA Moreno, B Trout, KA Sullivan, RA Roberts, RB Tjalkens.

39. Cellular Mechanisms Underlying Rapid Neuronal Osmoregulation. J Musser, Z Zhang, QQ Sun.

40. Pre- and postsynaptic regulation of proopiomelanocortin neurons via multiple opioid receptor subtypes. Reagan L. Pennock and Shane T. Hentges.

41. Functions for the neuronal P-body component HPat in the control of neural plasticity in Drosophila. Sarala J. Pradhan, Leslie M. Rozeboom, Mani Ramaswami, and Scott A. Barbee.

42. GLYT1 glycine transporter modulates NMDA receptor function in goldfish retina.
E Rozsa and J Vigh.

43. Effects of Copper on Synaptic Plasticity. NL Salazar-Weber, JP Smith.

44. Gene deletion of inducible nitric oxide synthase suppresses glial inflammation and protects against manganese neurotoxicity. Karin Streifel, Julie Moreno, Briana Trout, and Ron Tjalkens.

45. Glial interactions and neuroinflammation in Manganese neurotoxicity. Sullivan KA, Moreno JA, Ahrens JA, and Tjalkens RB.

46. Structurally diverse cationic neurotoxicants attenuate ATP-dependent calcium signaling in astrocytes. Briana Trout, Karin Streifel and Ronald Tjalkens.

47. D1/5 modulation of hippocampal circuits affects salience-associated learning and memory. Juan Varela.

Neuroendocrine

48. Rates of HPA axis habituation are similar in male and female rats despite acute sex differences. JA Babb, SK Sasse, CV Masini, S Campeau, HEW Day.

49. Investigation of seasonal AMP-activated protein kinase expression in golden-mantled ground squirrels (Spermophilus lateralis). J L Bateman, J E Healy, G L Florant, and R J Handa.

50. Monitoring Performance Degradation of Cerebellar Functions Using Computational Neuroscientific Methods: Implications on Neurological Diseases. RA Nawrocki, SE Shaheen, M Shaalan, NM Lorenzon.

51. Restraint stress rapidly induces MAP kinase phosphatase-1 expression in the hypothalamic paraventricular nucleus. C Osterlund, V Thompson, E Jarvis, RL Spencer.

52. Ligand dependent and independent activity of TAM family members in GnRH neuron development and reproductive function. A Pierce, M Xu, B Bliesner, G Lemke, J Richards, S Tobet, and ME Wierman.

53. Restoration of the GnRH system in aging male transgenic animals paired with females. JR Rochester, WCJ Chung, P-S Tsai.

54. Paraventricular Nucleus of the Hypothalamus: Novel Vascular Development and Relation to Adjacent Cell Types. Melanie Schow, J. Gabriel Knoll, Qian Zhang, Stuart Tobet.

55. Fear-potentiated startle induces c-Fos expression in serotonergic neurons selectively within the dorsal part of the mid-rostrocaudal dorsal raphe nucleus.
BM Spannuth, AK Evans, JL Lukkes, MW Hale, S Campeau, CA Lowry.

56. Urocortin 2 activates a subpopulation of ventricle-projecting serotonergic neurons in the dorsal raphe nucleus. CE Stamper, MW Hale, DR Staub, CA Lowry.

57. Exposure to an open-field in low-light and high-light conditions increases c-Fos expression in phenotypically distinct subpopulations of neurons in the rat basolateral amygdala. AM Westerman, MW Hale, CA Lowry.

58. Presence of GnRH system in the mouse heart. H. Talbott, D. C. Skinner, Tao-Yiao John Wu.

59. Regulation of stress-induced gene expression in the hypothalamic paraventricular nucleus (PVN) and anterior pituitary by an acute PVN corticosterone microinjection.
MJ Weiser, J Highland, RL Spencer.

Sensory and Motor Systems

60. Mu-opioid receptor and its preferential endogenous agonist β-endorphin are expressed in the mouse retina. J Angeln, J Mower, SK Gallagher and J Vigh.

61. Effect of carbon chain length, number and type on iron mediated lipid peroxidation of the brain, kidney, and liver. WE Briner.

62. Electrophysiology of Early Vision in Musca domestica. K. Creaser, L. Benson, E. Tomberlin, S. Barrett, C. H.G. Wright.

63. Effects of pretreatment with clozapine on spatial memory of rats with lesioned dorsal hippocampi. J Losacco, ME Basham.

64. Cerebellum-directed aprotinin infusions inhibit plasmin activity but do not impair rat motor learning. ME Basham, ML Skoch, EE Grange.

Cognition and Behavior

1. Effects of neonatal exposure to progesterone on the development and expression of asult male sexual behavior in the rat.

A.B. Breton, M.G. Leedy, K.J. Austin, and B.M. Alexander.
From the Department of Animal Science, University of Wyoming, Laramie, Wyoming

Treatment of at-risk pregnancies with exogenous progesterone increased almost two-fold from 2003 – 2006. Expression of progesterone receptors is sexually dimorphic during sexual differentiation of the fetal brain and may be influenced by exogenous hormone. The objective of this experiment was to determine the role of the progesterone receptor in the development of pathways that differentiate sexual behavior in the male. Neonatal male pups received RU486, P4, or vehicle (n = 7/group) postnatally d 1 - 5. Sexual behavior was evaluated at 10.5 wk. Although growth was similar among treatment groups (P = 0.98), testes weight was decreased in RU486 (P = 0.04) but not P4 (P = 0.69) males. At initial exposure to estrous females, intromissions (P = 0.07) and ejaculations (P = 0.02) were decreased in RU486 treated males, while intromissions in P4 treated males were intermediate between RU486 and control males and ejaculations were decreased (P = 0.02). Latency to first intromission was increased (P = 0.08) in RU486 treated males. Latency to first mount or ejaculation was not noted (P ≥ 0.14). Expression of sexual behavior did not differ (P ≥ 0.19) among treatment groups by the last test. No treatment by time interaction (P ≥ 0.68) was noted. Hypothalamic expression of P4 receptor during the treatment period was similar (P = 0.80) among untreated male and female littermates. Exogenous progesterone and the progesterone receptor antagonist (RU486) had similar inhibitory effects on initial expression of male sexual behavior. Antagonistic effects of progesterone may be due to down-regulation of its receptor, or RU486 may have an agonistic effect. Subsequent behaviors may be a result of an incomplete inhibition of the P4 receptor during development or a reflection of the positive reinforcement that successful mating provides.

Key Words: Male sexual behavior, Progesterone, Development

2. CSF Aβ40 and Aβ42 levels and cognitive dysfunction in the senior beagle.

Christina de Rivera[1,2], Joseph A. Araujo[1,2], Bill Milgram.[1,2]
[1]Department of Pharmacology, University of Toronto, Toronto, Canada
[2]CanCog Technologies Inc., Toronto, Canada

Dogs model human cognitive decline and pathological aging. With advanced age, dogs show deficits in memory, inhibitory control and attention. Among aged animals, individual differences in cognitive performance model those seen in aged humans and therefore provide canine models of mild cognitive impairment and dementia. Aged dogs also develop brain atrophy, ventricular enlargement, and accumulate human type β-amyloid (Aβ) in plaques with development occurring earliest in the prefrontal cortex. Like humans, the predominant species of Aβ in the dog brain is the longer, more toxic fragment Aβ1-42, rather than Aβ1-40, and the structure of both are identical to those in humans. The present study further extends the canine model by: 1) assessing cerebrospinal fluid (CSF) Aβ levels in groups of dogs varying in age; and 2) correlating serum and CSF Aβ levels to performance on tests of cognitive function. Lumbar CSF samples were collected from over 50 Beagle dogs from three age groups: young (2-4 years); old (6-8 years); and senior (≥ 10 years). Aβ40 and Aβ42 levels were measured using ELISA. Levels of Aβ40 did not differ significantly between the three age groups. By contrast, Aβ42 levels showed an inverted U-shaped relationship with age, with the senior animals showing significantly decreased levels when compared to the old animals ($p < 0.05$). We also measured plasma Aβ40; which increased progressively with age. These findings indicate a complex relationship between age and soluble Aβ, as detected in CSF. We suggest that the lower levels of Aβ42 seen in senior dogs correlates with cognitive impairment seen in advanced age and that the decreased levels reflect greater cortical aggregation of Aβ42 in senior dogs than in old or younger dogs. These findings are consistent with data from human subjects indicating lower Aβ42 levels in AD patients than in aged

matched controls. The current findings provide exciting new evidence of commonalties between human and canine cognitive aging.

3. Perturbation of Molecular Pathways underlying behavior.

M Ahemed, D Dubach, X Huang-Sturgeon, A Costa, K. Cios, C Nguyen and K.Gardiner.
University of Colorado Denver, Colorado and Virginia Commonwealth University, Richmond, Virginia

Down syndrome, resulting from an additional copy of human chromosome 21 (HSA21), is the most common genetic cause of mental retardation and intellectual disability. The Ts1Cje mouse model of DS is trisomic for 83 genes orthologous to HSA21 and displays a number of behavioral deficits. The Ts1Cje and Ts65Dn DS mouse models, were shown to be hypersensitive to the locomotor stimulatory effects of NMDA receptor channel blockers. A recent behavioral study demonstrated that acute injections of MK801, an NMDA receptor antagonist, improved the performance of Ts1Cje mice in the fear conditioning test with learning and memory levels comparable to their wild-type counterparts. Analysis revealed significant differences in the levels of phosphorylated and total proteins of the MAPK pathway in the cortex of Ts1Cje mice and euploid controls. However, significant within-group individual variations in the details of the molecular and behavioral responses were observed. Comparisons of protein levels in individual mice with their associated locomotor activity showed no significant correlation for any single protein. The complexity of multiple protein contributions might be amenable to analysis by Machine Learning Methods (MLM). In collaboration with Dr Krys Cios, four MLM algorithms were tested. Input data were the sets of protein values from the 32 mice used in our MK801 experiments (Ts65Dn, Ts1Cje and controls, +/- MK801 injection). Output data was validated by removing one protein profile and its associated behavior. Algorithms predicted the behavior of the one removed sample by analyzing the remaining data. The Naïve Bayes algorithm had an 84% prediction accuracy in correctly assigning the behavioral activity of an individual mouse based upon the protein profile. This shows that simple protein profiles can be used to establish a positive correlation with behavioral outcome and to select candidate proteins for major contributions. We propose to apply a similar approach to the analysis of protein profiles from Tc1 and Ts65Dn mice. This will allow prediction of subsets of proteins that most significantly discriminate genotype, thus indicating contributions to phenotype made by genes uniquely trisomic in Tc1 and Ts65Dn.
EXPERTISE: Reverse phase protein arrays, Western blotting, Behavior testing

4. Shared neural circuitry for selection and initiation of actions.

Gidon Felsen and Zachary F. Mainen.
From the Department of Physiology & Biophysics, U.C. Denver, Aurora, CO.

Many models of decision making assume that the selection and initiation of actions are mediated by distinct neural substrates. However, some studies suggest that the two processes share a common circuitry. We have recently shown that the superior colliculus (SC) is necessary for the normal initiation of contralateral locomotor responses in rats, akin to its well-known role in eye movements in primates. We now examine what role, if any, the SC plays in the formation or selection of such locomotor responses.
We trained rats to perform a delayed-response version of an olfactory-cued spatial choice task. In each trial of the task, the rat 1) sampled a binary odor mixture presented at a central odor port, 2) waited in the odor port for 500 or 1000 ms until a tone was presented, 3) made a locomotor response to the left or right reward port, and 4) received a water reward if the correct port was selected. The reward side was contingent on the dominant component of the odor mixture cue. We found that in trials in which the rat selected the preferred direction of the cell, firing rate increased soon after odor presentation – when the correct response can first be selected – and remained elevated until the response was initiated. Comparing the timing of activity between trials with 500 and 1000 ms delays supported the idea that changes in SC activity were more closely coupled to the selection, rather than the initiation, of movement. If the SC is involved in the formation of the decision rather than simply action initiation, one might expect to observe stimulus-dependence of

SC responses, as has been documented in the primate SC for eye movements. Indeed, we found that SC firing rates reflected the difficulty of the odor mixture discrimination: preference for movement direction during the pre-movement period was stronger for easy compared to difficult discriminations.
Together, these observations indicate that the SC is not only involved in action initiation but in linking the stimulus to the response. Thus, this study provides an example of a system in which the same neural circuits that carry out actions are also responsible for making decisions about those actions.
EXPERTISE: Behavioral testing, in-vivo electrophysiology

5. Evidence-Based Practice for Early Cognitive Impairment Detection on Older Individuals with Metabolic Syndrome.

PC Heyn and Onofrei L.
From the Department of Physical Medicine and Rehabilitation, School of Medicine, University of Colorado Denver, Aurora, CO.

Alzheimer's disease (AD) is the most common form of dementia, and it has been associated to diabetes and metabolic syndrome. Due to the high prevalence of diabetes and metabolic syndrome in the older population, it is important to detect cognitive decline at early stages to allow prompt initiation of therapy and counseling of the patients and family. Objective: The objective of this study is twofold: 1) to systematically analyze the literature to investigate the clinical significance of FDG-PET in the early diagnosis of mild cognitive impairment (MCI), and 2) based on the systematic review, we examine by a case study design the patterns of hypometabolism found in two older female subjects with metabolic syndrome and subjective memory complaints. Systematic Review Methods: A review of literature has been conducted, using PubMed. Predefined inclusion criteria was used to determine inclusion and analysis of the papers. Systematic Review Results: A total of 6 studies were included in the review. Cognitive decline was predicted with sensitivities ranging from 38% to 93%, specificities ranging from 62.5% to 97% and accuracies from 81.8 to 90% (N=6). Case Study Methods: Two older female patients (P1& P2) were evaluated by a comprehensive battery of neurological, memory, and laboratory tests. Case Study Results: Both patients had insulin resistance and MS. The neuropsychological evaluation indicated that both patients suffer from mild MCI. Conclusions: The results of the review show that due to the high values for specificity, sensitivity, and accuracy, as well as due to the consistent patterns of hypometabolism detected, FDG-PET is a good diagnostic tool and shows great promise as a clinical tool in the early detection of MCI. As for the case study, the FDG-PET scans of P1 and P2 showed different patterns of hypometabolism, suggesting the patients have two different types of MCI. Although the neuropsychological tests were close to normal ranges, the PET scan was able to detect patterns of hypometabolism—and thus reduced function, consistent with the subjective memory complaints. FDG-PET was more reliable in predicting early MCI than the neuropsychological tests, as suggested by previous studies.
EXPERTISE: behavior/cognitive testing

6. STEP mediates ethanol inhibition of NMDARs, long-term potentiation and fear conditioning.

T.R. Hicklin[1], Wu P.H.[2,3], Radcliff R.A.[4], Freund R.K.[6], Goebel-Goody S.M.5, Proctor W.R.[2,3], Lombroso P.J.[5], Browning M.D.[1,6]
From the [1]Program in Neuroscience, University of Colorado Denver Anschutz Medical Campus; [2]Department of Psychiatry, University of Colorado Denver Anschutz Medical Campus; [3]Department of Veterans Affairs Eastern Colorado Health Care System;[4]Department of Pharmaceutical Sciences, University of Colorado Denver Anschutz Medical Campus; [5]Child Study Center, Yale University School of Medicine, New Haven, CT; [6]Department of Pharmacology, University of Colorado Denver.

Acute alcohol-induced memory loss is thought to occur via inhibition of NMDAR-dependent long-term potentiation (LTP) in the hippocampus. We reported previously that ethanol inhibition of

NMDAR function and LTP is correlated with a reduction in the tyrosine phosphorylation of Tyr1472 on the NR2B subunit and that ethanol's inhibition of NMDAR fEPSPs is attenuated by a broad-spectrum tyrosine phosphatase inhibitor. These data suggested that ethanol's effect on the NMDAR and LTP involved an unknown protein tyrosine phosphatase (Alvestad, et al. 2003). The direct interaction of NMDARs and STriatal Enriched protein tyrosine Phosphatase (STEP), and studies showing a correlation between STEP activity and Tyr1472 dephosphorylation raised the possibility that STEP may be an important mediator of ethanol effects (Pelkey et al., 2002; Braithwaite et al., 2006). Here we demonstrated that the absence of STEP activity significantly blocks ethanol inhibition of NMDAR function and LTP. Moreover, ethanol inhibition of NMDAR currents can be rescued after restoration of STEP activity. Furthermore, Western blot analysis revealed ethanol induces a de-phosphorylation of tyrosine residues on the NR2B subunits of NMDARs from WT but not STEP KO mice. Finally we found ethanol's disrupting effects on fear conditioning are attenuated in STEP KO mice. Taken together, our data suggest that STEP mediates ethanol inhibition of NMDAR function via dephosphorylation of tyrosine site(s) on NR2B and lend support to the hypothesis that STEP is required for ethanol's amnesic effects.

7. An economical quantitative ratiometric method for detecting cocaine and benzoylecgonine for forensic and biological applications.

DC Cooper[1,2], B Cadle[1], KC Rasmus[1], A Hannum[2], MM Marinelli[3], LS Leverich[1]. [1] From the Institute for Behavioral Genetics; [2]Dept. of Psychology and Neuroscience, University of Colorado, Boulder, Boulder, CO. [3]From the Dept. of Cellular and Molecular Pharmacology. Rosalind Franklin University of Medicine and Science, Chicago. IL.

We describe a new quantitative ratiometric pixel density analysis (QRPDA) method for the detection of cocaine and its major metabolite, benzoylecgonine. QRPDA is an economical alternative to more costly methods, such as gas chromatography or high pressure liquid chromatography. We performed image analysis of commercially available dye-conjugated anti-cocaine/benzoylecgonine (COC/BZE) antibody test strips in response to a series of cocaine concentrations from 0.003 to 0.1 mg/ml in order to establish a standard curve. Images were acquired using a mobile phone camera or a web cam. Automated image analysis was accomplished with IMAGEJ, the freely available image analysis software program and custom JAVA based macros. Results from the QRPDA method indicate a highly sensitive limit of detection of 3 ng/mg and exponential decay function between 3 and 100 ng/ml. QRPDA is a low cost method requiring only inexpensive COC/BZE test strips, a commonly available web or mobile camera, a computer and IMAGEJ software. QRPDA is appropriate for the detection of COC/BZE in biological fluid, such as urine. Mice were injected intraperitoneally with a low dose of cocaine (3.5 mg/kg) and their urine was found to contain ~500 ng/ml COC/BZE. To further investigate these results, we will look for correlations of cocaine intake and urine levels when rats are allowed cocaine self-administration. Lastly, QRPDA has the potential to be used as a forensics tool. We are in the process of using QRPDA to measure trace amounts of cocaine on a random sampling of credit cards from individuals in Colorado. Trace amounts of cocaine have reportedly been found on US currency and similar results are hypothesized with credit card testing. It is concluded that QRPDA is a low-cost highly sensitive method for the detection of COC/BZE with forensic and biological applications.

Development

8. Characterization of the oxytocin system in fibroblast growth factor 8 hypomorphic mice.

LR Brooks, WCJ Chung and PS Tsai.
From the Integrative Physiology Department and Center for Neuroscience, University of Colorado, Boulder, CO.

Oxytocin (OT) is a neurohormone critical for milk ejection during lactation. The cell bodies of magnocellular oxytocin neurons are found in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus. Increasing evidence shows that fibroblast growth factor 8 (FGF8) signaling is important for the development of the neuroendocrine system that secretes

gonadotropin-releasing hormone (GnRH), a system that originates in the olfactory placode. However, it is currently unclear if FGF8 is also important for the development of neuroendocrine systems that originate within the brain, such as the magnocellular OT system. In this study, we used immunocytochemistry to examine the presence of OT immunoreactive (ir) neurons in mice hypomorphic for the FGF8 allele (FGF8 hypomorphs). Since homozygous FGF8 hypomorphs do not survive past postnatal day 0 (PN0), we analyzed exclusively animals at this age. Our results show that the magnocellular OT-ir neurons are present in the PVN of mice homozygous for the hypomorphic FGF8 allele, and the number of these neurons appeared comparable among wild-type (WT), heterozygous, and homozygous FGF8 hypomorphs. However, in homozygous FGF8 hypomorphs, numerous OT-ir cells were also found within the ependymal layer lining the third ventricle, a phenomenon not observed in heterozygous and WT mice. The cellular identity of these ectopic OT-ir cells is unknown, but they are large and morphologically similar to the magnocellular OT neurons located more laterally and dorsally. In sum, our study suggests that FGF8 signaling may be important for the proper expression of OT in the differentiated OT neurons. Alternatively, since magnocellular neurons originate within the ventricular neuroepithelium and migrate laterally to reach their final destination in PVN, FGF8 deficiency could cause the magnocellular OT progenitor cells to migrate aberrantly resulting in the improper positioning of these neurons within the ependymal layer. Sources of Support: NIH HD042634

9. Heart field reduction in embryos depleted for voltage-gated calcium channel b subunit CACNB2.

Yelena Chernyavskaya, Alicia Ebert, and Deborah Garrity
From the Department of Biology, Colorado State University, Fort Collins, CO.

Voltage-gated calcium channels (VGCCs) are oligomeric complexes composed of pore-forming CACNA subunits and several auxiliary proteins. Auxiliary CACNB subunits regulate VGCC electrophysiology and chaperone CACNA subunits to the cell membrane. In humans, mutations in CACNA subunits are associated with congenital cardiac arrhythmia, but the developmental functions of the CACNB subunits are poorly understood. To determine the contributions of CACNB2 to cardiac development, we depleted zebrafish embryos of CACNB2 transcripts using morpholinos. CACNB2 morphant heart fields contained fewer cells expressing cardiac markers, suggesting fewer cardiomyocytes were specified, or diminished survival. Cell proliferation at later stages did not compensate for this deficiency; heart tubes in morphants contained 30% fewer cardiomyocytes at 48 hpf, as looping and chamber morphogenesis progressed. Moreover, morphant heart tubes fragmented easily when placed under pressure, suggesting that cardiomyocyte adhesion was weakened. Previous work showed that mutations in CACNA that inactivate cardiac VGCCs lead to atrial fibrillation. In contrast, heart rhythm was normal in CACNB2 morphants, suggesting that other CACNB proteins may compensate for the depletion, providing intact VGCC activity. We are currently assaying whether CACNB2 phenotypes are mediated by loss of VGCC function, or by loss of other CACNB2:partner interactions. The latter possibility is intriguing in light of recent data suggesting that CACNBs, as MAGUK-family proteins, may interact with multiple protein partners via their SH3 or guanylate kinase domains.
EXPERTISE: in situ hybridization

10. Cell survival and proliferation are disrupted in the olfactory placode of fibroblast growth factor 8 hypomorphic mouse embryos.

WCJ Chung and P.S. Tsai.
From the Department of Integrative Physiology and Center for Neuroscience, University of Colorado at Boulder, Boulder, CO.

Gonadotropin-releasing hormone (GnRH) neurons are scattered throughout the mouse preoptic-hypothalamus and represent the most upstream neuroendocrine regulators of the hypothalamus-pituitary-gonadal axis. Previous studies showed that GnRH neurons are first detected in the mouse olfactory placode (OP) around embryonic day (E) 10.5-11.5. Recently, we showed that GnRH neurons failed to emerge from the OP of E11.5 mouse embryos hypomorphic for fibroblast growth

factor (FGF) 8. Upon closer examination, we found that the homozygous Fgf8 hypomorphic embryos possessed a morphologically distinct OP with normal primary invagination. However, the secondary recess that presumably gives rise to the vomeronasal organ and GnRH neurons was severely reduced or absent. To better understand the mechanism underlying this disruption, we studied OP morphogenesis in E10.5-11.5 Fgf8 hypomorphs. Because FGF8 is important for developmental cell survival, we examined whether the E11.5 OP in Fgf8 hypomorphs underwent aberrant levels of apoptosis. We found that the E11.5 OP contained very few apoptotic cells, and there were no gross differences in apoptosis between wildtype, heterozygous and homozygous Fgf8 hypomorphs. In contrast, the level of apoptosis in the OP of E10.5 homozygous Fgf8 hypomorphs was much higher when compared to wildtype and heterozygous counterparts. Therefore, a time-specific disruption of OP cell survival on E10.5 may underlie the altered OP morphology found in E11.5 homozygous Fgf8 hypomorphs. PCNA immunostaining, a cell proliferation marker, revealed fewer proliferating epithelial cells in the OP of E10.5 homozygous Fgf8 hypomorphs. This observation is consistent with reduced OP thickness and primary OP invagination in this phenotype. Future studies will address if apoptosis prior to E10.5 also contributes to OP abnormality in Fgf8 hypomorphs, and if Fgf8 deficiency leads to reduced rate of progenitor cell proliferation, which may ultimately result in the absence of GnRH neurons in Fgf8 hypomorphs.

11. Reliability of ERN in Children Performing a Visual Flanker Task.

PL Davies and WJ Gavin.
From the Department of Occupational Therapy, Colorado State University, Fort Collins, CO.

This study examined the test-retest reliability of the ERN in children. Fifty-three children ages 8 to 13 years (Mean age = 10.3, SD = 1.5, Males = 23) completed a visual flanker paradigm in each of two sessions one week apart. No significant difference was found between the ERN peak-to-peak amplitude for session 1 (Mean=7.5, SD=4.0) and session 2 (Mean=8.1, SD=4.7). Moderate reliability in ERN amplitude was found (Cronbach's Alpha = .54; ICC = .37, p = .003). The reliability was examined after adjusting for trial-to-trial latency variability using an adaptive Woody filter to improve signal-to-noise ratios. The mean ERN amplitudes increased for session 1 (Mean=13.6, SD=7.8) and session 2 (Mean=14.6, SD=7.1) but difference between sessions was not significant. After adjusting for latency variability minor improvements in reliability was found (Cronbach's Alpha = .58; ICC = .40, p = .001). To illustrate developmental differences, the 20 youngest children (< 9.6 years) revealed no reliability between session 1 and 2 (Cronbach's Alpha = -.01; ICC = -.003, p = .51). For these children the latency adjustment had a large impact on reliability (Cronbach's Alpha = .56; ICC = .39, p = .04). For the 33 older children, the reliability between session 1 and 2 was stronger (Cronbach's Alpha = .66; ICC = .49, p = .002) though the adjustment for the latency variability had a similar reliability levels as the younger children. The results suggest the reliability of ERN amplitude is moderate for children between 8 and 12 years of age and that reliability improves with age.
EXPERTISE: EEG, Behavior testing

12. Reliability of ERN in Adults Performing a Visual Flanker Task.

WJ Gavin and PL Davies.
From the Department of Occupational Therapy, Colorado State University, Fort Collins, CO.

Impairment in performance monitoring may result in problems in everyday functioning. A reliable measure of underlying brain processing related to performance monitoring may be helpful in studying clinical populations. The error-related negativity (ERN) has been associated with performance monitoring. The purpose of this study was to examine the reliability of the ERN in a visual flanker task. In the present study, 21 healthy young adults (Mean age = 23.5, SD = 2.5; 10 males) completed a visual flanker task in each of two sessions, one week apart. The ERN peak-to-peak amplitude for session 1 (Mean=11.6, SD=5.4) was not significantly different from the ERN amplitude for session 2 (Mean=13.2, SD=5.0), F (1, 20) = 3.2, p=.087. The reliability of the ERN amplitude between sessions was significant and very high (Cronbach's Alpha = .84; Pearson's r =

.72; ICC = .72, p <.0005). To improve the signal/noise ratio in the averaged ERP, an adaptive Woody filter was used to correct for trial-to-trial latency variability in the ERN amplitude for both sessions. After adjustment, the ERN peak-to-peak amplitude for session 1 (Mean=16.2, SD=5.4) was significantly different from the ERN amplitude for session 2 (Mean=18.7, SD=6.7), F (1, 20) = 10.3, p=.004. The reliability of the ERN between sessions improved after adjusting for latency variability (Cronbach's Alpha = .90; Pearson's r = .84; ICC = .82, p <.0005). These results suggest that the ERN amplitude is highly reliable in adults and the reliability improves after correcting for noise related to latency variability.
EXPERTISE: EEG/ERP

13. Calcium channel MAGUK gene CACNB4 required for mitosis in zebrafish early development.

Cory J. Harrell, Alicia M. Ebert, William B. Horne, Deborah M. Garrity.
From the Department of Biology, Colorado State University, Fort Collins, CO and College of Veterinary Medicine, Cornell University, Cornell, NY

Voltage-dependant calcium channels (VDCC's) are comprised of a pore forming alpha1 (CACNA) subunit and several auxiliary subunits. The CACNB auxiliary subunits chaperone the alpha1 subunit to the membrane and modulate gating properties of the channel. Mutations in the CACNB4 subunit are associated with ataxia and seizures in mice and with epilepsy in humans, but since known mutant alleles of CACNB4 are not embryonic lethal, the developmental functions of the protein are unclear. In studying the functional roles of the CACNB4 gene, we unexpectedly discovered that targeted knockdown of CACNB4 genes in zebrafish led to arrest or delay of epiboly and subsequent death of the early embryo. In CACNB4 knockdown blastula-stage embryos, we localized the phenotypic defects to the extra-embryonic yolk syncytial layer (YSL), a syncytium containing a few hundred nuclei. We find that nuclei in the YSL fail to remain physically separate, and instead form multipolar spindle arrays that fail mitosis. Supernumerary centrosomes appear to contribute to the inappropriate joining of adjacent nuclei. We also report progress on strategies to use transgenic lines to determine the subcellular localization of CACNB4 in the YSL, and to identify the CACNB4 domains required for normal epiboly.
EXPERTISE: in situ hybridization, morpholino injection, gateway cloning, RT-PCR, restriction digestion

14. Developmental Profile and Sexually Dimorphic Expression of Kisspeptin in the Mouse Brain.

JG Knoll, CM Clay, GJ Bouma, TR Henion, GA Schwarting, RP Millar, SA Tobet. Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523, University of Massachusetts Medical School, Worcester, MA 01655, and The Queen's Medical Research Institute, Edinburgh, UK.

The Hypothalamic-pituitary-gonadal axis (HPG) is a complex neuroendocrine circuit involving multiple levels of feedback. Kisspeptin neurons likely play essential roles in controlling the HPG axis from the perspectives of puberty onset, oscillations of gonadotropin releasing hormone (GnRH) neuron activity and the pre-ovulatory LH surge. In contrast to most studies that focus on the postnatal expression and function, the current studies investigated the expression of kisspeptin during murine fetal development using in situ hybridization (ISH) and quantitative reverse transcriptase real-time PCR (qPCR) for mRNA, and immunocytochemistry for peptide. Expression of kisspeptin and its receptor GPR54 mRNAs were seen at embryonic (E) day 13 by ISH. From E13 to adulthood, the kisspeptin mRNA signal in individual cells within the arcuate nucleus (ARC) appeared stronger in females than males. ISH examination of agonadal steroidogenic factor-1 (SF-1) knockout mice revealed that E17 male knockouts resembled wild-type females more than males. This finding implies that the expression of kisspeptin mRNA in the ARC is modulated by gonadal hormones prior to birth. The sex difference was tested quantitatively by qPCR experiments in dissected hypothalami from mice at E17 and adulthood. Females had significantly more kisspeptin mRNA than males at both ages, even though the number of cells detected by ISH

was similar. The detection of immunoreactive kisspeptin in perikarya of the ARC at E17 indicates that this early mRNA is translated to peptide. The function of this surprisingly early expression of kisspeptin mRNA awaits elucidation.
EXPERTISE: Immunocytochemistry, in situ hybridization, PCR, tissue culture, microscopy (fluorescent, brightfield, video, digital imaging), animal husbandry

15. Role of nitric oxide in the expression of gonadotrophin releasing and gonadotrophin inhibiting (RFamide related peptide) hormone in mouse development.

Pankaj Kumar[1], Kazuyoshi Tsutsui[2], Paul Huang[3], Stuart Tobet[1]
From the [1]Department of Biomedical Sciences, Colorado State University, Fort Collins, CO; [2]Department of Biology, Waseda University, Tokyo, Japan and [3]Cardiovascular Research center, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Nitric oxide (NO) has been implicated in various physiological functions, including brain development, cardiovascular regulation, and reproductive functions. Immunoreactive neuronal nitric oxide synthase (nNOS) has been demonstrated in neurons surrounding gonadotropin releasing hormone (GnRH) neurons and NO has also been suggested as a regulator of GnRH release. A role for NO in the regulation of RFamide-related peptide [RFRP; the mammalian homolog of gonadotrophin inhibiting hormone (GnIH)] in mammals is undetermined. The present study tested whether genetic disruption of nNOS affects the detection of immunoreactive GnRH and RFRP neurons in development. Paraformaldehyde fixed brains from postnatal day 0 WT and KO were sectioned coronally and processed for immunoreactive GnRH and RFRP in adjacent sections. The number of immunoreactive GnRH neurons in KO mice (171+22.67 in one third of the total number of sections) was significantly greater ($p=0.05$) compared to the WT (106+16.0). The difference was mostly attributable to neurons in the region of the organum vasculosum of lamina terminalis (OVLT). Immunoreactive RFRP neurons were counted from the plane of the paraventricular nuclei to mammillary nuclei. The majority of cells were located in the dorsomedial nuclei and again there were significantly more ($p<0.001$) immunoreactive RFRP in KO (569.75+23.7) than in WT (350.25+32.8). The RFRP immunoreactive fibres projected toward GnRH neurons in
the region of OVLT in both WT and KO even at this early age (P0). It is possible that NO alters the expression of both GnRH and RFRP early in development. The physiological role of this regulation and the mechanism of its generation awaits discovery.
EXPERTISE: Immunocytochemistry, behaviour testing, tissue culture

16. Enriched environments protect against depression brought about by chronic mild stress and increase neuronal density in the dentate gyrus in Sprague-Dawley rats.

McKenzie Letendre, ME Basham
From the Neuroscience Program, Regis University, Denver, CO

Enriched environments (EE) integrate complex housing conditions with social stimulation and are shown to ameliorate symptoms of depression in rats with as much success as pharmacological treatment. Furthermore, rearing in EE is associated with an increase in hippocampal neourgenesis. This study attempted to demonstrate the depression related behavioral effects of rearing in EE in contrast to rearing in social isolation (SI) using a sucrose preference test (SPT) and forced swim test (FST). I hypothesized that rats reared in EE would exhibit less anhedonia and behavioral despair during and after exposure to a chronic mild stress procedure and would show an increased density of neurons in the hippocampus. The rats were reared for 61 days in either EE or SI conditions and then exposed to chronic mild stress for 14 days and behavioral measures were taken during and after chronic mild stress. Upon completion of the behavioral study, three rats from each condition were sacrificed and neuronal density in the hippocampus was determined. I found that EE prevent behavioral despair demonstrated by the FST and that EE increase density of neurons in the hippocampus providing a possible mechanism for the behavioral effects of EE.

17. Determining the role of the Tbx5 transcription factor in zebrafish cardiac development.
LE Parrie, YA Chernyavskaya, DM Garrity.
From the Department of Biology, Colorado State University, Fort Collins, CO
Tbx5, a T-box transcription factor, is required for cardiac development. Mutations of Tbx5 lead to Holt-Oram Syndrome (HOS) in humans. As in HOS, mutation of zebrafish tbx5 affects both heart and forelimbs structures. Homozygous tbx5/ heartstrings (hst) exhibit bradycardia, failure of the heart tube to loop, cardiac edema and absence of pectoral fins. Here, we investigate the effects of tbx5 mutation on 1) cell proliferation in the developing heart tube, 2) volumetric growth of cardiomyocytes, and 3) chamber morphology, especially during heart tube stages. Previous tbx5 overexpression studies in chick and mouse demonstrated that Tbx5 provides a growth arrest signal that limits cardiomyocyte proliferation during chamber morphogenesis stages. In the converse experiment we find that loss-of-function mutation of zebrafish tbx5 did not lead to increased cardiomyocyte number, with no net effect on cell proliferation of heart tube cardiomyocytes at 48 or 72 hours post-fertilization. We hypothesize that inability of the hst heart tube to loop may result from deficiencies in cardiomyocyte shape, size or differentiation. We provide an update on ongoing work to determine the earliest developmental timepoints at which tbx5 is necessary for normal cardiac function, as well as the functional relevancy of graded tbx5 expression, by using the Tg(hsp70:tbx5-GFP) and Tg(cmlc2:tbx5-GFP) lines of zebrafish.
EXPERTISE: in situ hybridization, real time qPCR, zebrafish transgenesis

Disorders of the Nervous System

18. Overexpression of amyloid precursor protein induces mitochondrial oxidative stress and intrinsic apoptosis cascade.
Bartley, MG[1], Linseman, DA[1,2]
[1] University of Denver, Dept. Biological Sciences and Eleanor Roosevelt Institute, Denver [2] Veterans Affairs Medical Center, Research Service, Denver
Down syndrome (DS) is the most common genetic form of cognitive disability and is caused by trisomy of chromosome 21. Within the DS critical region of chromosome 21 is the gene, amyloid precursor protein (APP). Proteolysis of APP into toxic and aggregate-prone, beta-amyloid fragments underlies the pathophysiology of Alzheimer's disease (AD). Individuals with DS uniformly develop early onset AD; however, the role of APP overexpression in this comorbidity is controversial. Here, we elucidated the mechanism of cell death induced by overexpression of wild type APP. Chinese hamster ovary cells transfected with a DsRed-APP fusion construct displayed caspase-3 activation and nuclear fragmentation indicative of apoptosis. APP-induced apoptosis was blocked by a pan-caspase inhibitor, BOC, glutathione (GSH), or co-expression of Bcl-2. APP depleted mitochondrial GSH, induced opening of the permeability transition pore, and caused cytochrome c release. Each of these events was inhibited by GSH but was unaffected by BOC indicating that they were oxidative stress-dependent and upstream of caspases. We conclude that APP overexpression is sufficient to cause mitochondrial oxidative stress and intrinsic apoptosis. We are currently examining if a similar cell death pathway is induced by APP in neuronal cells. Our data are consistent with an increased expression of APP being a likely contributor to neuron death in DS. Thus, decreasing APP-induced oxidative stress and apoptosis may be beneficial in reducing the comorbid phenotype of DS patients afflicte d with AD. (Supported by a VA merit review grant and R01NS062766 from NINDS).
EXPERTISE: immunocytochemistry, sterile cell culture, transfection, transformation

19. Polymeric Nanowire Templates as Scaffolds for Improved Neuronal Cell Functionality.
SL Bechara, KC Popat.
From the School of Biomedical Engineering, Department of Mechanical Engineering, Colorado State University, Ft. Collins CO
Growth and maintenance of neural cells on surfaces with unique nanotopography can be beneficial in several tissue engineering applications including central nervous system regeneration, spinal cord injuries, etc. PCL is biocompatible as well as a biodegradable material that has the potential

to be used as a scaffold for a variety of tissue engineering applications. In this work, we investigated the effects of PCL nanowire surfaces on neuronal cell viability, adhesion, and differentiation. Viability as assessed by the MTT assay showed that after 1 and 4 days of culture, the viability for the cells adhered on the PCL nanowire surfaces was significantly higher than that of the control surfaces. This implies higher cell coverage on the nanowire surfaces contrasted by low cell coverage on both of the control surfaces. The nano-topography of the PCL nanowires allowed the PC12 cells to adhere at much higher densities than the control surfaces. This can be seen in fluorescence microscopy images of cells stained with Calcien. The most probable mechanism behind the increased cell coverage was enhanced integrin signaling and adhesion promoted by the nanotopography of the nanowire surfaces.
After terminally differentiating the PC12 cells by exposing them to NGF, a number of visualization techniques were utilized to assess cell morphology and coverage post-differentiation. CMFDA fluorescent images showed that the nanotopography present in the PCL Nanowires promoted high cell adhesion and extended neurite formation. The SEM images reveal extensive grouping and neurite formation on the nanowire surfaces as well as confirm the fluorescent adhesion results. Immuno-fluorescence staining for Tyrosine Hydroxylase and Neurofilament-H was also preformed to confirm neural differentiation. This work demonstrates that by manipulating nano-topography, PC12 cells can be effectively cultured onto a surface and the material can actually increase growth, differentiation, and adhesion. Opportunities to further this research in neural tissue engineering could ultimately lead to an improved material for bio-scaffolding and stem cell therapies.
EXPERTISE: immunocytochemistry, flow cytometry, tissue culture, sterile technique

20. Neuroadaptations in mesolimbic dopamine neurons mediate avoidance behavior to social defeat and antidepressant actions.
JL Cao, M Wilkinson, HE Covington III, EJ Nestler, DC Cooper and MH Han.
From Department of Psychology and Neuroscience, Institute for Behavioral Genetics, University of Colorado, Boulder, CO.
The ventral tegmental area (VTA) dopamine neurons are involved in mediating stress-related psychological disorders. Here, we report that chronic social defeat increases in vivo spontaneous firing rates and bursting events companied with the increased hyperpolarization-activated cation currents (Ih) and decreased GABAergic synaptic input in VTA dopamine neurons, all of which are normalized by chronic, not acute, treatment with fluoxetine, a antidepressant, indicating that neuroadaptations in VTA dopamine neurons mediate avoidance behavior to social defeat and antidepressant actions.

21. Characterization of the Adel Mutant of Chinese Hamster Ovary Cells: A Cellular Model of Adenylosuccinate Lyase Deficiency.
N Duval, LK Vliet, TG Wilkinson II, GN Vacano, D Patterson.
Eleanor Roosevelt Institute and Department of Biological Sciences, University of Denver, Denver, CO
Adenylosuccinate lyase (ADSL) is an enzyme that catalyzes two non-sequential steps in the de novo synthesis of AMP; the conversion of succinylaminoimidazole carboxamide ribotide (SAICAR) to aminoimidazolecarboxamide ribotide (AICAR) and the conversion of succinyl AMP (AMPS) to AMP. Clinically, ADSL mutations lead to an enzyme deficiency characterized by psychomotor retardation, autistic features, hypotonia, and seizures. Hypotheses regarding the pathogenesis include toxicity of high levels of SAICAR, AMPS, or their metabolites, lack of ability to synthesize sufficient purines using the de novo purine biosynthetic pathway. One important approach in developing an understanding of ADSL deficiency is through the development of cell culture models. These models will allow for the study of ADSL deficiency and different ADSL mutant properties at the cellular level, and may also help in the development of effective treatments. We previously reported isolation and initial characterization of mutants of Chinese hamster ovary (CKO-K1) cells (Ade I) that could be used for this purpose. Ade I cells lack detectable ADSL

activity, accumulate SAICAR and AMPS, and require adenine for growth, which is caused by a valine to alanine substitution at amino acid position 291 (V291A). This missense mutation occurs in the "signature sequence" of ADSL and causes inactivation of the enzyme and gene silencing. Here we show the expression levels of CHO-K1 and AdeI by reverse transcriptase PCR (RT-PCR) and quantitative PCR (qPCR) analysis. qPCR analysis shows that the AdeI mutant has a two-fold decrease in ADSL mRNA expression levels when compared to CHO-K1.Enzyme activity assays of ADSL lysates show ADSL inactivation in AdeI. RT-PCR analysis with human ADSL (hADSL) specific primers of CHO-K1, and AdeI transfected with mutant hADSL show that mutant ADSL can be effectively transfected into the AdeI cells and used to study different mutations in vivo.

22. Selectivity of the protective effects of docosahexaenoic acid (DHA) in the N2A neuronal cell line.

ML Florez-McClure, AE Garrison, and CM Butt.
From Discovery Neuroscience, Martek Biosciences Corporation, Boulder, CO. Docosahexaenoic acid (DHA) is vital in neurodevelopment, and decreases in brain DHA levels are associated with neurodegeneration. However, the mechanisms through which DHA promotes brain health have not been fully elucidated. DHA protects neurons from apoptosis through activation of Akt, Raf, and retinoid X receptors, but reactive oxygen species derived from fatty acids have been associated with decreased viability. These findings suggest that DHA may exhibit some selectivity in its maintenance of protective mechanisms. We sought to address this question by evaluating DHA's protection of the N2A neuronal cell during insults with okadaic acid (OA). Assays of cell viability (XTT), Hoechst staining, propidium iodide (PI) incorporation, and mitochondrial function (TMRE) were employed. DHA was protective against deprivation of polyunsaturated fatty acids in the XTT, Hoechst, and TMRE assays. DHA also conferred protection from OA in the XTT, Hoechst, and TMRE assays, but the degree of protection was assay dependent. The XTT and TMRE assays indicated significantly greater viability and mitochondrial function in cells treated with OA+DHA than cells treated with OA alone. Interestingly, comparisons between the OA and OA+DHA treatment groups in the Hoechst and PI assays did not detect significant differences. Measurements of apoptosis with caspase-3 expression and of reactive oxygen species with dichlorofluoroscein are currently underway. The current findings coupled with previously reported data suggest that DHA's protection of N2A cells is more anti-apoptotic than anti-necrotic and that promotion of mitochondrial function is involved.
EXPERTISE: immunocytochemistry, tissue culture, behavior, fluorescent assays, confocal microscopy

23. RNA interference against hypoxia-upregulated 1 (Hyou1).

DL Jost, A Poczobutt, S Jones, K Jonscher, D Leake, C Yamada, L Barrows, M Das, WM Zawada.
University of Wyoming, Laramie fd
Oxidative and endoplasmic reticulum (ER) stresses are considered key contributors to the pathogenesis of Parkinson's disease. To examine which proteins play a role in the pathophysiological events in dopaminergic neurons during the progression of the disease, we performed a comparison of protein expression using a proteomic approach in neurotoxin MPP+ treated immortalized dopaminergic cell line, N27. MPP+ treatment induced oxyradical production in these cells. Proteins were then resolved on 2-D gels and differentially expressed proteins were isolated and identified using mass spectrometry. The MPP+ treatment resulted in upregulation of several different proteins, including a member of HSP70 (heat shock protein) family. We then focused our investigation on one member of the HSP70 family, Hypoxia upregulated 1 (Hyou1, or ORP150). Hyou1 is an ER protein which regulates apoptotic responses of cells.
We hypothesized that altering Hyou1 expression levels will affect susceptibly of dopaminergic neurons to stressors. Using an RNAi approach, we tested three different Accell siRNA sequences with predicted Hyou1 knockdown in Neuro2a cells. These siRNAs have improved stability and have been modified for lipid-free cell penetration. The Hyou1 siRNA that produced nearly total knockdown in vitro was then selected for use in vivo knockdown testing. Hyou1 siRNA and

nontargetting control (NTC) or siGLO lamin A/C fluorescently-labeled control were infused into the dorsal 3rd ventricle for two weeks using Alzet osmotic pumps. After the two-week infusion, control siRNA was found to be distributed throughout the brain with the most prominent accumulations along the ventricular system and throughout the hippocampus. Immunocytochemistry revealed partial knockdown in the hippocampus and potentially other nuclei. We conclude that the use of stable siRNA preparations in osmotic pumps might be a useful tool for exploring ER stress in models of neurodegeneration.

24. Neuroprotective effects of anthocyanins on mitochondrial oxidative stress induced neuronal death.

NA Kelsey, WA Hulick, and DA Linseman.
University of Denver, Dept. Biological Sciences and Eleanor Roosevelt Inst., Denver Veterans Affairs Medical Center, Research Service, Denver

Neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS) are devastating diseases that affect both the patient and the people around them. A major cause of these diseases is mitochondrial oxidative stress (MOS) leading to neuronal death. In this study, we investigated the neuroprotective effects of a class of nutraceuticals, anthocyanins, against MOS-induced death using rat cerebellar granule neurons (CGNs). Anthocyanins are antioxidant compounds whose mechanism of action is yet to be elucidated. Kuromanin and callistephin, derived from black rice and strawberries, respectively, are the anthocyanins we have been investigating. HA 14-1, an inhibitor of the pro-survival and antioxidant protein Bcl-2, which selectively increases oxidative stress in mitochondria, was used to evoke MOS-induced death in CGNs. In a previous study, another natural antioxidant, epigallocatechin 3-gallate (EGCG), proved to be neuroprotective in this paradigm. Therefore, we used EGCG and glutathione (GSH) to compare the effectiveness of callistephin and kuromanin at protecting CGNs from MOS induced death. Preliminarily, both callistephin and kuromanin appear better at protecting CGNs from MOS-induced death than EGCG, but neither is as effective as GSH treatment. HA 14-1 treatment alone resulted in approximately 90% apoptosis of CGNs and both callistephin and kuromanin reduced this effect to approximately 20% cell death. Measurement of mitochondrial GSH levels in CGNs revealed that, HA 14-1 reduced this pool of GSH to 40% of the control mitochondrial GSH. Callistephin and kuromanin blocked this
reduction of this crucial pool of mitochondrial GSH. These data indicate that callistephin and kuromanin may be viable neuroprotective agents for therapeutic development in the treatment of neurodegenerative diseases caused by MOS. (Acknowledgments: Funding was provided by a VA merit review grant and 1R01NS062766 from NINDS)
EXPERTISE: western blotting, immunocytochemistry, cell culture

25. Iron Accumulation in Mouse Huntington's Disease Brain Occurs in Neurons.

E. Marks[a], K. Dorsey[b], Steven Hersch[b], Barry Lai[c], Jonathan Fox[a].
[a]Graduate Neuroscience Program and Department of Veterinary Science, University of Wyoming, WY [b]MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, MA. [c]Argonne National Laboratory, Argonne, IL

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a glutamine expansion within huntingtin protein. We have previously shown that iron levels are elevated early (pre-clinically) in the pathogenesis of mouse HD. However, little is known about where iron elevation occurs in HD brain tissue and the underlying molecular mechanisms involved. Further, it is unknown whether appropriate modulation of iron metabolism is neuroprotective in HD patients. We evaluated the spatial distribution of iron (II/III) in HD mouse brain by synchotron-based micro-x-ray fluorescence. Compared to wild-type litter-mate controls we found that HD brain had increased iron within neuronal perikarya as discrete puncta, a distribution that is consistent with the reported distribution of macroautophagy vesicular components. We also used the Perl's iron (III) stain but found no differences between wild-type and HD mice, suggesting that the iron accumulation we have found is in the 2+ oxidation state and not bound to ferritin (3+ oxidation state). In parallel

studies we delivered the iron chelator deferoxamine into the ventricles of HD mice and demonstrated protective effects as measured by behavioral and quantitative pathology outcomes. Together our results indicate accumulation of iron within HD neurons and protective effects of iron chelation. Mutant huntingtin expression results in activation of macroautophagy in HD neurons. We hypothesize that there is secondary accumulation of iron in this pathway due to breakdown of iron-rich proteins. The down-stream effects of this are iron-mediated oxidative damage to autolysosomes and also functional deficiency elsewhere within the neuron. We outline some of the experiments planned to address this hypothesis and present our neuronal culture model that we plan to use in these studies.

26. Cardiovascular and functional benefits of acupressure following stroke.

KL McFadden, S Huerta, TD Hernández.
From the Department of Psychology and Neuroscience, University of Colorado, Boulder CO.

Acupressure (i.e., stimulation of acupoints on the skin using fingertips rather than needles) has been implicated as a successful treatment for a variety of medical disorders, although underlying mechanisms remain unclear. One theory is that stimulation of acupoints modulates autonomic nervous system activity. Previous studies have suggested that acupressure may positively affect heart rate and blood pressure. The current study investigated the effects of a type of acupressure, Jin Shin, on cardiovascular function and physical activity in stroke survivors, a population that could especially benefit from a treatment promoting cardiovascular health. Our placebo-controlled study tested the hypothesis that active acupressure treatments would reduce heart rate and blood pressure (i.e., induce a greater relaxation response) and improve physical activity levels above and beyond that seen during placebo acupressure treatments. A randomized, placebo-controlled, single-blind crossover design was utilized, in which participants received 8 weeks of either active or placebo acupressure treatments followed by washout and crossover into the opposite condition. Heart rate and blood pressure measurements were taken throughout individual treatments, and physical activity levels were assessed before and after the 8 weeks of active and placebo treatments. Active acupressure treatments were associated with a significantly faster reduction in heart rate compared to that seen during placebo treatments ($p = .002$), suggesting an increased relaxation response. Moderate physical activity levels were significantly greater following active acupressure treatments than placebo treatments ($p = .023$). No treatment effect on blood pressure was found. A functional consequence of the increased relaxation response observed during active treatments may be reflected in the significant increase in physical activity levels seen following active acupressure treatments. Although no treatment effect on blood pressure was found, this could be due to 67% of participants taking medication to control their blood pressure during the study.

EXPERTISE: Human neuropsychological testing

27. Characterization of cerebellar Purkinje cells harboring a malignant hyperthermia mutation (Y522S) in the intracellular Ca2+ release channel, RyR1.

Jason A. Santiago, George C. Talbott, Nancy M. Lorenzon.
Department of Biological Sciences. University of Denver, Denver, CO, USA

Calcium ions perform an essential function not only in the electrical activity of cells, but also in diverse downstream cellular signaling. Calcium levels must be precisely regulated both within distinct localized domains and more globally throughout a single cell. Indeed, the devastating consequences of calcium dysregulation are exemplified by the skeletal muscle disease malignant hyperthermia (MH). MH is a life-threatening disease in which halogenated anesthetics or depolarizing muscle relaxants trigger massive calcium release resulting in skeletal muscle rigidity, rapid rise in body temperature, and cardiac arrhythmias that are potentially lethal. MH results from altered intracellular calcium signaling due to mutations in the ryanodine receptor channel (RyR1). Mouse models have recently been generated and characterized (Chelu et al. 2005). Skeletal muscle harboring the Y522S-RyR1 knock-in mutation exhibit Ca2+ leak from internal stores, an increase in reactive oxygen and nitrogen species, basal cellular stress, and ultimately progressive

mitochondrial and cellular damage (Durham et al. 2008). Although the alterations in the calcium release channel function itself and resultant changes in cellular function have been characterized in skeletal muscle, the effects of MH mutations in RyR1 on central nervous system function have not been investigated. We have initiated studies investigating 3 main aspects of Y522S-RyR1 Purkinje cells: intracellular calcium release, cellular organization/morphology, and cellular stress. In preliminary studies using imaging techniques with the calcium indicator dye Fura2, RyR-mediated calcium release in Y522S-RyR1 Purkinje neurons exhibited a negative shift in the apparent EC50 for the agonist caffeine. In addition, intracellular calcium store content was decreased in Y522S-RyR1 neurons. Since calcium signaling is important during neuronal development and maturation, the morphology of Y522S-RyR1 Purkinje cells was examined using immunohistochemistry and confocal microscopy. Moreover, we have initiated studies to determine if altered RyR1 function results in basal cellular stress using Western blot and immunohistochemical analyses.
EXPERTISE: Confocal Microscopy, Calcium Imaging, Immunohistochemistry

28. Reovirus myelitis and glial activation.

SA Schittone and KL Tyler.
Microbiology Department, University of Colorado, Aurora, CO.

We have recently developed a model of viral induced spinal cord (SC) injury, in order to better understand the basic mechanisms of injury and develop novel treatment strategies. Neonatal mice are infected with 10^6 plaque forming units of a neurotropic reovirus (T3) in the right hind limb. This leads to a progressive paralysis first of the ipsilateral hindlimb and then of both hindlimbs (paraplegia). Tissue was collected from paralyzed animals for use in western blot (WB) analysis, immuno-fluorescence analysis (IFA) with confocal microscopy, or RNA isolation for gene expression analysis using reverse transcriptase PCR (RT-PCR) – evaluating levels either on agarose gel or quantitatively by syber green based real time PCR (qPCR). Viral infection was associated with an increase of glial fibrillary acidic protein (GFAP) + astrocytes in proximity to infected neurons in the anterior horn of lumbar spinal cord, as well as activation associated cell hypertrophy. Microglial activation was also evident as determined using IFA with antibodies against ionized calcium-binding adaptor molecule 1 (Iba1). Microglia showed altered morphology consistent with activation with quantitative cell counts showing an 18 fold increase in activated microglia in SC from paralyzed as compared to control mice. Activated microglia were found throughout the lumbar spinal cord and in close association with infected neurons. Since both astrocytes and microglial contribute to host innate immune responses in the CNS through activation of cytokine and chemokine signaling pathways, we examined expression of genes encoding these molecules. We found significant upregulation of mRNA encoding many interferon regulated genes. We confirmed these increases at the protein levels for two key intermediaries involved in pathogen associated molecular pattern (PAMP) recognition, MyD88 (toll like receptor signaling molecule myeloid differentiation primary response gene 88) and Rig-I (anti-viral RNA helicase retinoic acid inducible protein I). In conclusion, we find that of the spinal cord in an experimental model of viral myelitis results in activation of both astrocytes and microglia in proximity to infected neurons and that this process is associated with up-regulation of key regulators of innate immunity including IFN-associated genes and PAMP recognition molecules.

29. Signal transducer and activator of transcription-5 plays a novel role in neuronal apoptosis induced by inhibition of Rac GTPase.

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

In several neuronal cell types, the small GTPase Rac is essential for survival. Moreover, the ALS2 gene which is mutated in juvenile-onset amyotrophic lateral sclerosis codes for alsin, an exchange factor (GEF) for Rac. Inhibition of Rac with either C. difficile toxin B (ToxB) or an inhibitor of the Rac-specific GEFs, Tiam1 and Trio (NSC23766), induced apoptosis in primary cerebellar granule neurons (CGNs). Consistent with activation of a pro-apoptotic Janus kinase (JAK)/signal

transducer and activator of transcription (STAT) pathway, a pan-JAK inhibitor protected CGNs from Rac inhibition. STAT1
was induced by ToxB; however, CGNs from STAT1 knock-out mice succumbed to ToxB as readily as wild-type CGNs. STAT3 underwent tyrosine phosphorylation (PY) following ToxB and a reputed inhibitor of STAT3, cucurbitacin, reduced CGN apoptosis. Unexpectedly, cucurbitacin did not block STAT3 PY and CGNs were not protected from ToxB by other STAT3 inhibitors. In contrast, STAT5 PY induced by ToxB was blocked by cucurbitacin. In addition, roscovitine similarly inhibited STAT5 PY and protected CGNs from ToxB. Finally, adenoviral infection with a dominant negative STAT5 mutant, but not wild type STAT5, significantly decreased ToxB-induced apoptosis of CGNs. These data indicate that a novel JAK/STAT5 pro-apoptotic pathway contributes to neuronal apoptosis induced by inhibition of Rac GTPase. Identification of STAT5 target genes in Rac-inhibited neurons may provide new therapeutic avenues for neurodegenerative diseases involving loss of Rac function. (Supported by a VA merit
review grant).

30. Regulation of MCT1 Function By Intracellular pH and PKA Agonists in Cerebrovascular Endothelial Cells.

AL Uhernik, C Tucker, JP Smith.
From the Department of Biology, Colorado State University-Pueblo, Pueblo, CO

Monocarboxylic Acid Transporter 1 (MCT1) is expressed on the luminal and abluminal membranes of cerebrovascular endothelial cells where it is the sole transporter of monocarboxylic acids across the plasma membrane. Because monocarboxylates, such as lactic acid, are important brain energy substrates and play important roles in brain pathologies, such as stroke, understanding factors that regulate MCT1 protein function is important for brain health and disease. In this study, we describe two distinctly different mechanisms of regulating MCT1 in rat cerebrovascular endothelial cells. The first mechanism was changes in the intracellular pH which altered the driving force for lactate-proton co-transport. Cells treated with NBD-Cl, a V-Type ATPase inhibitor, or colchicine, a microtubule disrupting agent, had significantly lower and higher cytoplasmic pH values that were associated with slower and faster MCT1 kinetic function, respectively. Because acidic cytoplasmic pH values blocked lactic acid transport, it is possible that acidification of cerebrovascular endothelial cells accounts for the failure of MCT1 to prevent lactic acidosis in stroke brain, and alkalinization of the cytoplasm could present a new therapeutic approach. The second mechanism of regulating MCT1 was by treatment with membrane permeant cAMP analogs. Twenty minute exposure to 500 µM 8-Bromo-cAMP modulated MCT1 transport function by a mechanism that did not involve changes in cytoplasmic pH. We have previously shown that this is mediated by beta adrenergic signaling through a pathway including Protein Kinase A. The regulation was stimulatory, or inhibitory, depending upon the duration of cell culture and required a morphing of the actin cytoskeleton. Therefore, it is possible that cerebrovascular endothelial cells respond to hormones or neurotransmitters by changing their capacity to transport monocarboxylic acids across the blood brain barrier. This could have important consequences in normal brain energy metabolism and may be relevant in pathological lactic acidosis of the brain.
EXPERTISE: immunostaining, epifluoroscence microscopy, cell culture, and ratiometric imaging using pH-dependent fluorescence microscopy

31. A novel role for Bcl-2 in the regulation of mitochondrial glutathione transport.

HM Wilkins, J Schwarz, and DA Linseman.
Dept of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver, Colorado, USA; Veterans Affairs Medical Center, Denver, Colorado, USA

Mitochondrial oxidative stress (MOS) is a major factor in neuronal cell death that underlies various neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS). Aberrant mitochondrial production of reactive oxygen species and the resulting oxidative damage within these organelles are ameliorated by endogenous antioxidant defenses of which glutathione (GSH), is a key player. Moreover, GSH is synthesized exclusively in the cytoplasm and must be actively transported into

mitochondria. Specific depletion of the mitochondrial GSH pool is known to sensitize cells to MOS, and could lead to novel therapies in neurodegeneration. Recently, two anion carriers localized to the inner mitochondrial membrane(IMM), the dicarboxylate (DIC) and 2-oxoglutarate (OGC) carriers, have been identified as principal mitochondrial GSH transporters in liver and kidney; GSH transport in neurons has not been investigated. Here, we show that Bcl-2, a suppressor of MOS, is a key regulator of the mitochondrial GSH pool in primary cultures of rat cerebellar granule neurons (CGNs). Incubation of CGNs with a Bcl-2 inhibitor, the BH3 mimetic HA14-1, induced MOS and caused specific depletion of the mitochondrial GSH pool. Bcl-2 expressed in lysates from transfected Chinese hamster ovary (CHO) cells was captured onto GSH-agarose beads and was antagonized by the BH3 mimetic. Finally, Bcl-2 was co-immunprecipitated with OGC from co-transfected CHO cells, an interaction which was enhanced by GSH and inhibited by HA14-1. Our data indicate that Bcl-2 is a GSH-binding protein and a possible interacting partner for the IMM OGC. GSH-binding (likely within the BH3 groove) may enhance the affinity of Bcl-2 for OGC and in this manner, may regulate the transport of GSH via the OGC across the IMM. We are currently investigating the role of the OGC and DIC in mitochondrial GSH transport in CGNs and whether Bcl-2 is a direct interacting partner for these transporters in neurons. We conclude that Bcl-2 acts as a key regulator of the mitochondrial GSH pool in neurons, a property which significantly contributes to its ability to suppress MOS and neuronal cell death. Supported by grants from the NINDS and VA.
EXPERTISE: Western Blotting, immunocytochemistry, immunoprecipitation

Neural Excitability, Synapse and Glia

32. Microglial response in the nucleus of the solitary tract after taste nerve damage.

DL Bartel, TE Finger.
Department of Cellular & Developmental Biology and Neurosciences Program, University of Colorado Denver Anschutz Medical Campus, Aurora, CO

The chorda tympani (CT) is an entirely sensory branch of the seventh cranial nerve and innervates taste buds on the anterior two thirds of the tongue. The CT is prone to injury during dental procedures and inner ear surgeries but is also highly successful in reinnervating taste buds in the 2-4 weeks following damage. Despite this regenerative proclivity and reappearance of taste buds, long-term taste abnormalities occur in the in patients following CT injury. Whether central reorganization occurs in the nucleus of the solitary tract (nTS) -- where the chorda tympani nerve fibers first synapse in the brainstem -- is not resolved. Peripheral nerve damage in other sensory systems can provoke central changes within glial populations in the first sensory relay. We sought to test whether microglia in the adult nTS respond to unilateral transection of the CT.

In adult mice, the CT was sectioned in its course via the middle ear. The mice survived for various times (2-3, 5, 10, 15, 20 and 30 days; n=4,5 at each time). Antibody staining for microglia using Iba1 (cytoplasmic calcium binding protein) shows a dramatic increase in the number of stained microglia in the nTS ipsilateral to nerve injury at 2 days post lesion and resolves to normal levels around four weeks. In contrast, there was no apparent response from astrocytes, as assessed with GFAP. Microglial cell counts on the uninjured side of the nTS are not significantly different from control levels while the number of immunoreactive microglia on the injured side are increased two to three times base-line levels. On the uninjured side, as in sham operated animals, Iba1 staining reveals microglia with thin, highly ramified processes and smooth nuclei indicative of a resting state. On the injured side, microglia in the nTS have short, thick processes and a more ‘ragged’ nuclei, a morphology typical of activated microglia.

We are studying possibilities that microglia proliferate locally in the nTS, are being recruited from other nearby nuclei or are perhaps being coming from the blood. Insights into origins and eventual function of these microglial cells and whether they are involved in synaptic remodeling will be useful for understanding CT injury-induced changes to the nTS.

EXPERTISE: Immunocytochemistry, histochemistry, fluorescent & brightfield microscopy, LSCM confocal, cell counts, surgical techniques

33. Knockout of TRPC5 channels in adult prefrontal cortex reduces burst-triggered depolarization and enhances cocaine reward.

DC Cooper, MA Fowler, M Hu, L Li, A Dietrich, L Birnbaumer.
From the Institute for Behavioral Genetics, Dept Psychology and Neuroscience, University of Colorado, Boulder, CO.

Drug addiction is a disease that is influenced by both genetic and environmental factors that result in altered excitability in the key brain regions associated with reward and decision-making. The prefrontal cortex (PFC) processes reward-related information; and pathologies in PFC excitability resulting from prolonged drug use may lead to the loss of control over drug intake associated with drug addiction. We show that layer 5 pyramidal neurons in the PFC exhibit a prolonged depolarizing response to Gq-coupled receptor activation, which produces a period of heightened excitability of the cell following brief bursts of action potential activity. This burst triggered delayed depolarization enables the cell to convert subthreshold inputs into persistent firing output and may be a way for the cell to hold information in a short term memory buffer. The delayed after-depolarization (dADP) is reduced by dopamine and chronic cocaine, which may serve to bias the cell towards very strong inputs, such as those associated with drug cues, while preventing the cell from responding to smaller, subthreshold inputs. The dADP is induced by activation of Gq-coupled receptors, such as metabotropic glutamate receptors or muscarinic acetylcholine receptors and is mediated by subsequent activation of a non-selective cation channel, which pharmacological data suggested to be a canonical transient receptor potential (TRPC) channel. We used in situ hybridization, immunoblots, and real-time PCR to examine the expression of the TRPC channels and found dense expression of TRPC5 in the pyramidal cell layers of the PFC. Using adeno-associated viral mediated knock-down of TRPC5 in the prefrontal cortex of TRPC5flx mice, we show that TRPC5 channels are necessary for induction of the dADP in the PFC. We show that loss of TRPC5 in the PFC increases the locomotor activating and rewarding effects of cocaine. Knock-out of TRPC1 channels, on the other hand, has no effect on the dADP and does not alter behavioral responses to cocaine, suggesting that TRPC5 homomultimeric complexes rather than TRPC1/5 heteromultimeric complexes underlie the dADP in the PFC.

34. BDNF dependent synaptic plasticity underlies some forms of long-term memory

GP Cortese, TR Chapman, RM Barrientos, SF Maier, and SL Patterson.
From the Department of Psychology & Neuroscience, University of Colorado, Boulder, CO.

BDNF dependent synaptic plasticity appears to play a critical role in the processes underlying some forms of long-term memory. Thus, it's been suspected for some time that perturbations of BDNF production or signaling might contribute to the development and or progression of aging-associated cognitive decline. The available evidence suggests that basal levels of both BDNF and its receptor TrkB do not change very much as a result of aging alone. We hypothesize that the combined effects of aging and a challenging life event may significantly decrease BDNF signaling and BDNF-dependent synaptic plasticity in the hippocampus. We have previously reported that 24-month-old rats show profound and specific deficits in the ability to form long-term memories after a brief experimentally induced bacterial infection; 3-month old rats do not. We have now extended these observations, using hippocampal slices from these animals to examine for the first time the combined effects of aging and a recent infection on several forms of synaptic plasticity. We have found that the specific deficits in long-term memory are mirrored by specific deficits in long-lasting forms of synaptic plasticity. The infection does not compromise the initial learning of the test tasks, or the formation of short-term memories in any of the animals. Similarly, the infection does not significantly compromise basal synaptic transmission, or short-term synaptic plasticity. In contrast, theta burst L-LTP, a BDNF-dependant form of long-lasting synaptic plasticity, was dramatically reduced in aged animals with a recent history of infection. In the work presented here, we have begun to examine the effects of age and infection on the production and processing of the BDNF protein. Whole hippocampal tissue was collected and used to prepare synaptoneurosomes. Here we report that BDNF protein levels are significantly reduced in synaptic fractions of hippocampal tissue from aged animals following bacterial infection. In addition, BDNF-associated synaptic

receptors are not affected by age and infection. These findings are important in understanding the functional role of BDNF and its influence on synaptic plasticity. These studies should provide further insight into the early stages of synaptic failure characteristic of many neurodegenerative disorders.
EXPERTISE: Western Blot, Tissue Culture, Electrophysiology, & Differential Centrifugation

35. Expression of importin ß-1 in the rat hypothalamus and its involvement in the nuclear transport of the membrane, neurokinin 3 receptor.

DD Jensen, SM Lyden and FW Flynn.
Graduate Neuroscience Program, Zoology & Physiology Dept. University of Wyoming

The tachykinin NK3 receptor (NK3R) is a G protein coupled receptor that is expressed by neurons in a variety of brain regions. We demonstrated previously that the NK3R is trafficked from membrane to nuclei of neurons in the paraventricular nucleus of the hypothalamus (PVN) in response to an osmotic challenge. There is no information regarding the mechanism for the nuclear transport of the NK3R with the exception of a putative nuclear localizing sequence (NLS) found on the NK3R. This NLS provides for the possible interaction of the NK3R with importins during nuclear translocation. The majority of the importin experiments have been done in in-vitro models and there is little information regarding the expression or function of importins in the brain. These experiments utilized: 1) immunohistochemistry to identify the presence of importin ß-1 in the hypothalamus and if it overlapped with the expression of NK3R in the hypothalamus and, 2) immunoprecipitation to determine if NK3R associated with importin ß-1 following activation. Male Charles River rats (300-350 g) were perfused with PBS and fixed with paraformaldehyde. Sections were processed for importin ß-1 and NK3R immunoreactivity. Analysis of sections showed that importin ß-1 was heavily expressed throughout the PVN and overlapped with the expression of NK3R. A separate group of rats were given an IV injection of hypertonic saline (2 M) that has been shown to activate the NK3R and cause transport to the nucleus, or isotonic saline as control. 40 min. after the infusion, the PVN was removed and homogenized. These samples were then tested to see if importin ß-1 co-immunoprecipitated with the NK3R. The importin ß-1 was co-immunoprecipitated with the NK3R following activation by hypertonic saline injection, but not following isotonic saline treatment. This is the first in-vivo study showing a link between importin ß-1 and the nuclear transport of the NK3R. This study shows that after internalization following a physiological challenge the NK3R is transported to the nucleus via the same pathway as many steroid receptors and transcription factors. (Supported by P20 RR15640 and NS 57823 to FWF.)

36. Dopamine D1 receptor activation induces synaptic plasticity via a novel NR2B-dependent mechanism and promotes memory consolidation.

LS Leverich, CE. Lane, JA Varela, RW Green and DC Cooper.

Dopamine D1-class receptor (D1R) activation modulates glutamate-dependent neuroplasticity thought to underlie learning and memory. Disturbances in dopamine-glutamate signaling have been implicated in many neuropsychiatric disorders. Despite its importance, the mechanisms mediating D1R modulation of glutamate-dependent synaptic plasticity require further characterization. Here we present evidence using field potential recordings from hippocampal slices showing that D1R activation establishes a prolonged temporal window for the induction of NMDA receptor-dependent synaptic plasticity. We found that D1R activation induces early-phase synaptic potentiation and increases long-term potentiation (LTP) expression through a pathway involving NR2B-NMDARs, Src-family tyrosine kinases, PKA, PKC, and PKMζ. D1R activation produced sustained increases in the surface expression of NR2B and GluR1 subunits in hippocampal slices but this effect is blocked by a selective NR2B antagonist. Consistent with the synaptic results, D1R activation facilitated the memory consolidation phase of an extinction learning task, thus providing behavioral relevance for a prolonged window of synaptic potentiation mediated by D1Rs. Furthermore, these results suggest that by enhancing extinction learning, D1R

activation may be a useful therapeutic approach for treating psychiatric disorders. EXPERTISE: Electrophysiology

37. The avian ciliary ganglion: a useful model for the study of beta amyloid effects on neurotransmitter release.

Susan Mazalan, Elizabeth Hart, and D. Bruce Gray.

Studies from this lab show that beta-amyloid peptide (1-42) (Abeta) can aggregate to a form that is able to disrupt synaptic cholinergic transmission between cultured avian ciliary ganglion neurons or between these neuronal terminals in the choroid layer of the embryonic eye. The cellular pathway for this effect involves cyclin-dependent kinase 5 (Cdk5) activity as well as nitric oxide (NO) induced activation of cyclic GMP dependent protein kinase (PKG). Unlike reports from hippocampal slices, the site of action of this neuromodulation is presynaptic, affecting evoked acetylcholine release. We also present data here suggesting that this pathway requires microglial cell activation due to the sensitivity of the beta amyloid effect to minocycline and inhibition of evoked Ach release is accompanied by an increase in interleukin 1-beta. The form of beta amyloid that is responsible for inhibition of Ach release appears to be soluble oligomers of more that 100 kDa as evidenced by dynamic light scattering and microfiltration measurements. These effects are distinct from cell death since these neurons can survive extended exposure to these oligomers and maintain high affinity choline uptake. Conflicting reports in the literature showing Abeta-induced synaptic facilitation or depression in different preparations may be resolved by the observation that exposure to the oligomer can also increase basal transmitter release while inhibiting evoked release.

38. Sub-Pathologic doses of MPTP stimulate glial activation in nuclear factor kappa B (NF-kB) reporter mice.

JA Miller, DL Carbone, JA Moreno, B Trout, KA Sullivan, RA Roberts, RB Tjalkens.
Center for Environmental Medicine, Program in Molecular, Cellular, and Integrative Neuroscience, Colorado State University, USA; Astra-Zeneca, Inc., Department of Safety Pharmacology, Alderly Park, UK

Parkinson's disease (PD) results from the progressive and irreversible degeneration of dopaminergic neurons. Although the etiology of the disease is not completely known aberrant neuroinflammatory stimulation is believed to be involved. In the present study we investigated glial specific inflammatory activation in correlation with neurobehavioral and neuropathological outcomes in a chemically induced parkisonian mouse model employing sub-pathologic doses of MPTP. Significant alterations in neurobehavioral and gait disturbances were observed upon exposure to MPTP with depression
of locomotor activity at day 1 post-treatment and reduction in hind-limb stride length on day 6 post-treatment at the highest dose (30 mg/kg) employed. No significant loss of dopaminergic neurons in the substantia nigra was apparent at any of the doses of MPTP employed as assessed by stereological counting of tyrosine hydroxylase positive cells. In contrast, quantification of nerve terminal tyrosine hydroxylase in the striatum showed significant reduction at 30 mg/kg with an associated reduction in dopamine and dopamine metabolite levels in the striatum. Assessment of glial activation was accomplished using a transgenic reporter mouse expressing green fluorescent protein (GFP) upon activation of the NF-kB signaling pathway. Significant increases in GFP expression was observed in GFAP positive astrocytes at all doses which correlated to the expression of the prototypic inflammatory protein NOS-2 at 7 and 15 mg/kg. In addition to increased NF-kB activation andNOS-2 expression an increase in 3-nitrotyrosine levels in the substantia nigra was observed at the two higher doses. Collectively these data demonstrate significant inflammatory glial activation at low to moderate doses of MPTP subsequent to overt neuropathology which may precede subtle neurobehavioral alterations. These data suggest that glial activation may represent an early event in the pathogenesis of PD offering a potential therapeutic target for halting the progression of the disease.

39. Cellular Mechanisms Underlying Rapid Neuronal Osmoregulation.

J Musser, Z Zhang, QQ Sun.
From the Department of Zoology and Physiology, Graduate Neuroscience Program
University of Wyoming, Laramie, WY

Osmotic regulation is crucial to vertebrate survival and is one of the most tightly controlled homeostatic parameters. Severe or rapid hyponatremia can result in cerebral edema, which is a major cause of morbidity and mortality following various types of brain injury and stroke. Despite the sophistication and effectiveness of systemic osmoregulation, hormonal corrective mechanisms are slower than the osmotic challenges encountered. Cerebrospinal fluid can undergo rapid osmotic fluctuations between initiation of an osmotic challenge and the restoration of osmotic set-point. Cellular protection mechanisms are necessary to prevent or compensate for osmotically-induced changes in cell volume. This study investigates the cellular response to and molecular mechanisms of hypoosmotic detection in neurons. In neonatal brain slices, acute hypotonic challenge by local water application induces a robust, rapid and reproducible increase in intracellular [Ca2+] as measured by Fura-2 calcium imaging. The rapid [Ca2+]i increase is followed by a slow and nearly complete recovery (Tau = 6s and 135s respectively, N=9). Low frequency (5s challenge/5min) hypotonic challenge caused repetitive reversible responses with partial desensitization (60% decrease). Higher application frequencies overwhelm cellular Ca2+ homeostasis mechanisms and lead to an irreversible increase in [Ca2+]i. Preliminary electrophysiological data indicates a 20% decrease in membrane capacitance. Size measurements from GFP imaging confirm volume reduction. Pharmacological investigations showed that the Ca2+ response is not blocked by TTX, Cadmium, Gadolinium, AACOCF3, KB-R7943, U73122, or MRS1845. The Ca2+ signal is reduced by LOE-908 (broad-spectrum cation channel antagonist), Ruthenium Red (ryanodine activated Ca2+ release antagonist), and Suramin (inhibitor of receptor/G-protein coupling and purinergic receptors). Ca2+-free aCSF blocks the Ca2+ increase or significantly reduces it if cells are first challenged in normal aCSF. This indicates that the Ca2+ response is initiated by a receptor or stretch activated plasma membrane channel and sustained by Ca2+ release from intracellular stores. Elevated intracellular Ca2+ then initiates a rapid regulatory volume decrease which protects the cell from swelling.
EXPERTISE: Calcium Imaging, In Vitro Slice Preparation

40. Pre- and postsynaptic regulation of proopiomelanocortin neurons via multiple opioid receptor subtypes.

Reagan L. Pennock and Shane T. Hentges.
Department of Biomedical Sciences, Colorado State University,

Proopiomelanocortin (POMC) neurons release peptide transmitters including the endogenous opioid beta-endorphin that affect food intake, the HPA-axis, nociception, and the reward response. Thus, it is important to understand how the activity of these neurons is regulated. An autoregulatory system has been proposed since these neurons release opioids and express mu opioid receptors which mediate inhibitory postsynaptic responses. However, opioids also inhibit presynaptic transmitter release onto POMC neurons. The aim of the present study was to determine which subtypes of opioid receptors mediate pre- and postsynaptic actions of opioids in POMC neurons. Whole cell voltage clamp recordings were made in brain slices prepared from transgenic mice with labeled POMC neurons to analyze the effect of the selective opioid receptor agonists U69593 (kappa), DPDPE (delta), and DAMGO (mu) on pre- and postsynaptic regulation of POMC neurons. All three agonists reduced the amplitude of the evoked and spontaneous GABA mediated inhibitory postsynaptic currents (IPSCs). Application of U69593 and DAMGO resulted in the reduction of the amplitude of evoked glutamate mediated excitatory postsynaptic currents (EPSCs), but DPDPE had no effect. Only the mu receptor-specific agonist DAMGO induced an inhibitory postsynaptic current. The EC50 for DAMGO induced inhibition of IPSCs was ~50 nM whereas the EC50 for the postsynaptic outward current was ~360 nM. The differential expression and sensitivity of pre- and postsynaptic opioid receptors could have important

implications for the regulation of POMC neurons and beta-endorphin release in response to endogenous opioids or in response to opioid administration.
EXPERTISE: Whole Cell Voltage Clamp

41. Functions for the neuronal P-body component HPat in the control of neural plasticity in Drosophila.

Sarala J. Pradhan[1], Leslie M. Rozeboom[1], Mani Ramaswami[2], and Scott A. Barbee.[1] [1]Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver CO [2]Smurfit Institute of Genetics and TCIN, Lloyd Building, Trinity College Dublin, Dublin-2, Ireland.

The establishment of long-term memory (LTM) has been shown to require local protein synthesis in dendrites. Local mRNA translation is controlled by sequence motifs within mRNAs acting in concert with specific mRNA binding proteins and microRNAs (miRNAs). Together, the composition of these ribonucleoprotein (RNP) particles determines whether mRNAs are transported to a specific location (e.g. to the synapse), locally translated, or targeted for storage and/or degradation. New evidence suggests that dendritic RNPs in Drosophila neurons share highly conserved translational repression machinery with cytoplasmic RNA processing bodies (P-bodies). Initially thought to be sites of mRNA decay, P-bodies have since been shown to be involved in both general- and miRNA-mediated translational repression pathways. Here, we focus on the function of HPat, a highly conserved P-body protein involved in translational repression, P-body assembly, and mRNA decapping.Using primary cultures of Drosophila motor neurons, we have found that HPat localizes to Staufen (Stau)- and Fragile X Mental Retardation Protein (dFmr1)-containing neuronal RNPs. Additional studies indicate that HPat is a dominant modifier of dFmr1 function in the developing eye and is a negative regulator of synapse size at the larval neuromuscular junction (NMJ). Together, our results suggest that HPat has essential functions in the control of plasticity processes in Drosophila neurons.

42. GLYT1 glycine transporter modulates NMDA receptor function in goldfish retina.

E Rozsa and J Vigh.
From the Department of Biomedical Sciences, College of Veterinary and Biomedical Sciences, Fort Collins CO.

Beside its well-characterized role as an inhibitory neurotransmitter, glycine is a co-agonist of NMDA receptors: glycin binding is necessary both for ion channel opening and receptor internalization. The concentration of glycine in the synaptic cleft is regulated by the glycine transporters GLYT1 and GLYT2. The close spatial association of GLYT1 and glutamatergic synapses across the CNS strongly supports a role for this protein mediating NMDA receptor function via inluencing their glycine binding site occupancy. In the retina, GLYT1 is localized on glycinergic amacrine cells (ACs), whereas NMDA receptors mediate glutamatergic excitation of ACs and ganglion cells (GCs). The objective of the present study was to elucidate the possible role of glycine transporter 1 (GLYT1) in modulating NMDA receptor function in the retina.
Experimental approach: Goldfish retinal slices were prepared at room temperature in daylight conditions. Short, focal NMDA-puff evoked currents were recorded from voltage clamped ACs, GCs and bipolar cell (BC) terminals in the presence of glycine receptor blocker strychnine. ALX 5407, a selective and potent inhibitor of GLYT1 was bath applied. Short, focal glycine-puff evoked currents were recorded from voltage clamped ACs. Results: Application of ALX 5407 increased NDMA receptor mediated currents in ACs and GCs. NMDA puff evoked GABA release from ACs, which in turn triggered IPSCs in BCs. ALX 5407 also increased these NMDA-evoked IPSCs in BCs. In the presence of strychnine, glycine puff evoked glycine transporter-mediated currents in ACs, which was sensitive to ALX 5407, but not to ALX 1393 (GLYT 2 blocker).
Conclusion: GLYT1 localized on glycinergic ACs influences NMDA receptor function in the retina as well as elsewhere in the CNS.
EXPERTISE: patch clamp, retinal slice recording

43. Effects of Copper on Synaptic Plasticity.

NL Salazar-Weber, JP Smith.
From the Department of Biology, Colorado State University, Pueblo, CO

Copper is found in some synapses at levels around 100 μM, yet the physiological relevance of this is unknown. In some neurodegenerative diseases such as Menkes, Wilson's, and Alzheimer's disease, mis-regulation of copper is part of the pathological process and affects glutamatergic systems involved in excitotoxicity and synaptic plasticity. We studied copper's effects on two forms of synaptic plasticity in the CA1 region of mouse hippocampal slices: Long-term potentiation (LTP), and Paired Pulse Facilitation (PPF). CA1 LTP is a type of long-lasting synaptic strengthening that is mainly dependent on post-synaptic NMDA-type glutamate receptor signaling. PPF is a form of short-term plasticity that is mainly pre-synaptic. Both LTP and PPF are thought to be important mechanisms in learning and memory. We have found that 5 μM copper in the recording bath inhibited LTP but did not significantly alter PPF. However, one hour after induction of LTP we found that copper significantly enhanced PPF. This was shown to require LTP and was not simply the result of an extended incubation in copper. We will test the hypotheses that 1) copper exerts its effects on LTP through a direct interaction with post-synaptic NMDA-receptors; 2) copper inhibits LTP through enhanced release of neurotransmitter leading to desensitization of AMPA-receptors; 3) copper's effects on PPF are due to a direct pre-synaptic mechanism; and 4), copper's effects on PPF are through a mechanism involving post-synaptic retrograde signaling to the pre-synaptic terminal. It is important to elucidate the mechanism by which copper effects synaptic plasticity since this is currently unknown, and, our work could have implications for the diagnosis and treatment of Alzheimer's disease and other neuropsychiatric disorders involving copper.
EXPERTISE: Extracellular CA1 field potential recording in mouse hippocampus.

44. Gene deletion of inducible nitric oxide synthase suppresses glial inflammation and protects against manganese neurotoxicity.

Karin Streifel, Julie Moreno, Briana Trout, and Ron Tjalkens.
Department of Environmental and Radiological Health; Program in Molecular, Cellular and Integrative Neurosciences, Colorado State University, Fort Collins, CO

Overexposure to the essential nutrient manganese (Mn) can lead to a degenerative neurological disorder termed manganism. Reactive gliosis and subsequent neuroinflammation are implicated in the progression of the disease, with selective increases in reactive nitrogen species associated with neuronal injury. Previous studies from our laboratory demonstrated that expression of inducible nitric oxide synthase (NOS2) is increased in astrocytes in mice subchronically exposed to Mn and that this increase associates with elevated levels of 3-nitrotyrosine (3NT) protein adducts in neurons, a marker of nitrosative stress. Based upon these data, we postulated that mice lacking NOS2 would be protected against the neurotoxic effects of Mn and exhibit decreased gliosis and neuronal protein nitration. We therefore utilized NOS2 knockout ($Nos2^{-/-}$) mice to determine if loss of this gene protects against Mn neurotoxicity *in vivo* and *in vitro*. Mice were exposed to $MnCl_2$ daily by intragastric gavage from d21-d34 postnatal and examined for changes in locomotor function, glial activation, and neuronal protein nitration. Our results indicate protection of a variety of locomotor deficits in $Nos2^{-/-}$ versus wildtype (WT) which were found to have significant increases in total and rearing movements with treatment, however $Nos2^{-/-}$ mice showed no change to control in these perimeters. Histopathological scoring of glia indicated a trend to decrease in activation of astrocytes and microglia from Mn-treated $Nos2^{-/-}$ mice. Utilizing co- immunofluorescence it was found that a decrease in 3NT production in neurons located in the basal ganglia of the $Nos2^{-/-}$ Mn-treated mice. Additionally, *in vitro* co-culture studies revealed that $Nos2^{-/-}$ primary astrocytes activated by treatment with Mn and TNFα induced apoptosis in primary striatal neurons, but that this apoptotic phenotype was attenuated in neurons incubated with $Nos2^{-/-}$ astrocytes. These data demonstrate that NOS2 expression in glia is causally involved in Mn-induced neurotoxicity in developing mice and suggest that neuronal protein nitration may be an important determinant of neuronal injury from Mn. EXPERTISE: immunofluorescence, co-culture

45. Glial interactions and neuroinflammation in Manganese neurotoxicity.

Sullivan, KA[1], Moreno, JA[1], Ahrens, JA[1], and Tjalkens, RB[1,2].
[1]Environmental Health and Radiological Sciences, Colorado State University, Fort Collins, CO, USA [2]Cellular and Molecular Biology, Colorado State University, Fort Collins, CO, USA

Microglia are the resident immune cells of the brain that help protect the brain from stress and infection but chronic activation of microglia results in the production of chemokines and proinflammatory mediators such as tumor necrosis factor α (TNFα) and inducible nitric oxide synthase (NOS2). Previous studies have implicated activated microglia and astrocytes in neurodegenerative disorders of the basal ganglia such as Parkinson's disease and manganism. Recent work from our laboratory identified activated microglia expressing NOS2 in the basal ganglia of mice exposed to manganese (Mn) before the appearance of activated astrocytes; however, the mechanism by which Mn activates microglia and the role of activated microglia in manganism is poorly understood. In this study we postulated that Mn directly activates microglia, resulting in increased expression of TNFα and iNOS that ultimately lead to increased astrocyte activation. Primary microglia and astrocytes were isolated from C57Bl/6 mice in mixed cultures then purified for experiments. Immunofluorescence staining for microglia and astrocytes was performed using ionized calcium binding adaptor protein-1 (IBA-1) and glial fibrillary acidic protein (GFAP), respectively. This method yielded culture purities of 97% for microglia and 99% for astrocytes. Treatment of microglia with Mn induced a dose-dependent expression of TNFα and NOS2 mRNA and protein. Furthermore, a quantitative PCR array revealed increased expression of proinflammatory mediators in microglia when treated with Mn. Treatment of astrocytes with conditioned media from Mn-treated microglia or via co-culture with microglia caused an activated phenotype characterized by increased iNOS expression. Collectively, these data indicate that Mn activates microglia in a dose-dependent manner resulting in increased production of proinflammatory mediators that enhance activation of astrocytes, suggesting a complex pattern of glial-glial interactions underlying a neuroinflammatory phenotype in this model.

46. Structurally diverse cationic neurotoxicants attenuate ATP-dependent calcium signaling in astrocytes.

Briana Trout, Karin Streifel and Ronald Tjalkens.
Center for Environmental Medicine, from the Department of Environmental and Radiological Health, Colorado State University, Fort Collins, CO Program in Molecular, Cellular and Integrative Neurosciences, Colorado State University, Fort Collins, CO

Calcium signaling throughout networks of astrocytes is initiated by synaptic activity and in order to increase regional cerebral blood flow (rCBF) in response to the local demand for oxygen and glucose. This increase in intracellular calcium $[Ca2+]_i$, in perivascular astrocytes causes a release of several vasoactive factors from cellular endfeet contacting arterioles that causes a rapid dilation of the vessel. Deprecations in rCBF are well described in patients with Parkinson's disease (PD) but the mechanisms underlying these decreases are unknown. To examine the possible contribution of astrocyte dysfunction to this phenomenon, we postulated that several structually diverse neurotoxicants of the basal midbrain, all of which are cationic, would inhibit transmitter-induced calcium signaling in culture astrocyutes: MPP+, the active metabolite of the model parkinsonian neurotoxicant, 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP);Paraquat (PQ); 6-Hydroxydopamine (6-OHDA); and Manganese (Mn^{2+}). Using calcium imaging in primary cultured cortical astrocytes, we investigated the effect of acute treatment with each neurotoxicant on ATP-induced intracellular calcium transients. We observed a dose dependent decrease in ATP-induced $[Ca2+]_i$ transients with acute application of PQ, 6-OHDA and MPP+. In addition, mechanically-induced intercellular $[Ca2+]_i$ waves were inhibited in the presence of MPP+, an effect that was reversible following washout of the compound. Like MPP+, PQ, 6-OHDA, and Mn^{2+} similarly inhibit $[Ca2+]_i$ waves, however Mn^{2+} requires a higher concentration to produce equivalent calcium wave inhibition. These findings indicate that endogenous and exogenous chemicals that are structurally diverse but that have cationic properties inhibit physiological calcium signaling in astrocytes. Because these astrocytic signals are critical to regulation of rCBF, these data suggest a new target

for neurotoxicants that may provide insight into mechanisms of decreased cerebral blood flow in PD.
EXPERTISE: Calcium Imaging, cell culture

47. D1/5 modulation of hippocampal circuits affects salience-associated learning and memory

Juan Varela.
University of Colorado at Boulder, CO.

Converging evidence suggests that salience-associated modulation of behavior is mediated by the release of monoamines and that monoaminergic activation of D1/5 receptors is required for normal hippocampal-dependent learning and memory. However, it is not understood how D1/5 modulation of hippocampal circuits can affect salience-associated learning and memory. We have observed in CA1 pyramidal neurons that D1/5 receptor activation elicits a bi-directional long-term plasticity of NMDA receptor-mediated synaptic currents with the polarity of plasticity determined by NMDA receptor, NR2A/B subunit composition. This plasticity results in a decrease in the NR2A/NR2B ratio of subunit composition. Synaptic responses mediated by NMDA receptors that include NR2B subunits are potentiated by D1/5 receptor activation, while responses mediated by NMDA receptors that include NR2A subunits are depressed. Furthermore, these bidirectional, subunit-specific effects are mediated by distinctive intracellular signaling mechanisms. As there is a predominance of NMDA receptors composed of NR2A subunits observed in entorhinal-CA1 inputs and a predominance of NMDA receptors composed of NR2B subunits in CA3-CA1 synapses, potentiation of synaptic NMDA currents predominates in the proximal CA3-CA1 synapses, while depression of synaptic NMDA currents predominates in the distal entorhinal-CA1 synapses. Finally, all of these effects are reproduced by the release of endogenous monoamines through activation of D1/5 receptors. Thus, endogenous D1/5 activation can, 1) decrease the NR2A/B ratio of NMDAR subunit composition at glutamatergic synapses, a rejuvenation to a composition similar to developmentally immature synapses, and, 2) in CA1, bias NMDA receptor responsiveness towards the more highly processed tri-synaptic CA3-CA1 circuit and away from the direct entorhinal-CA1 input.
EXPERTISE: Patch Clamp

Neuroendocrine

48. Rates of HPA axis habituation are similar in male and female rats despite acute sex differences.

JA Babb, SK Sasse, CV Masini, S Campeau, HEW Day.
Dept of Psychology, University of Colorado at Boulder. CO.

It is uncertain to what degree gender variations in stress responses might predispose women to certain psychiatric illnesses such as major depression and some anxiety disorders. We have previously shown that sex differences in hypothalamic-pituitary-adrenal (HPA) axis responses can vary depending on the stressor. Specifically, female rats show robustly higher adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels in response to acute restraint stress, but similar magnitude response to acute noise stress as compared to male rats. It is unclear whether females would show the same rate of habituation to repeated presentations of these stressors. To test this, intact male and female Sprague-Dawley rats were presented with 30 minutes of either 100 dBA white noise or restraint each day for 10 days. Blood samples were obtained via a nick in the lateral tail vein of each animal on days 1, 3, 6, and 10. Another group of animals received the same handling and blood sampling but did not receive either stressor (no stress controls). Plasma was then analyzed for ACTH and CORT concentration. Overall, female rats that received no stress had significantly higher basal levels of both ACTH and CORT compared to male rats that received no stress, consistent with previously published literature. Noise stress significantly increased ACTH and CORT levels on Day 1 for both male and female rats. For both sexes, ACTH and CORT levels in response to noise stress habituated in parallel, with responses for both hormones back to no stress control levels by Day 6. Restraint stress also increased CORT levels on Day 1, and females

had a significantly higher response compared to males, as we had shown previously. However, although females had a more robust acute response, the rate of habituation across days did not differ significantly in males and females, as CORT levels in both sexes returned to baseline by Day 10. These data show that male and female rats do not have different rates of habituation in HPA axis responses following repeated stress regardless of the existence of an acute sex difference in these responses. This study also suggests that acute stress responses do not necessarily predict the ability of the organism to adapt to repeated presentations of that stressor.
EXPERTISE: estrous cycle monitoring

49. Investigation of seasonal AMP-activated protein kinase expression in golden-mantled ground squirrels (Spermophilus lateralis).

J L Bateman, J E Healy, G L Florant, and R J Handa.
From the Department of Biology, Colorado State University, Fort Collins, CO and Department of Basic Medical Sciences, University of Arizona College of Medicine, Phoenix, AZ. AMP-activated protein kinase (AMPK) is activated in response to high levels of AMP in a cell. Consequently, AMPK initiates catabolic and inhibits anabolic pathways. It is also implicated in the food intake pathway since increased AMPK activity leads to changes in hypothalamic arcuate (ARC) neuron activity. The golden-mantled ground squirrel is a mammalian hibernator which goes into torpor throughout the winter months. During this time, food intake is suppressed. Since AMPK plays a strong role in food intake regulation, it may also be important in food intake suppression during hibernation. We investigated whether AMPK expression in the ARC differs between the summer 3-day fasted and summer fed states using immunohistochemistry (IHC). We hypothesized that neurons of summer 3-day fasted animals would express more AMPK than neurons of summer fed animals. Our preliminary IHC results show a trend towards higher active AMPK levels in fasted animals, but significance was not reached. IHC has also been performed on brain slices from winter-state ground squirrels, including both torpid and euthermic animals. We hypothesized that torpid squirrels would show lower AMPK expression than winter euthermic animals due to suppressed enzyme activity during torpor. Our results show that the two groups were not significantly different. Finally, AMPK expression in summer state animals was compared to AMPK expression in winter. We found that there is more AMPK expression in summer compared to the winter state. We conclude that there are changes in AMPK expression between summer and winter, but once
animals enter the winter state, AMPK expression is not altered by torpor. This work was supported by NIH NS039951 grant to RJH and NIH R25DK067017 to GLF.

50. Monitoring Performance Degradation of Cerebellar Functions Using Computational Neuroscientific Methods: Implications on Neurological Diseases.

RA Nawrocki[1], SE Shaheen[2] , M Shaalan[3], NM Lorenzon[4].
From the [1]Department of Computer Engineering, University of Denver, Denver, CO, [2]Department of Physics and Astronomy, University of Denver, Denver, CO, [3]Department of Computer Science, University of Denver, Denver, CO, [4]Department of Biological Sciences, University of Denver, Denver, CO. This research presents computational analysis of cerebellar function in relation to neurological diseases affecting either a specific cell type, such as granule cells or Purkinje cells, or more generally affecting cerebellar cells, and the implications on effects in relation to performance degradation throughout the progression of diseases. The modeled cerebellar circuitry included mossy fiber and climbing fiber inputs, granule, Golgi and basket cells, interacting with Purkinje cells which project out of cerebellar cortex to deep cerebellar nuclei. The results show that, in our model, the overall number of cells of a particular type and primarily their proximity to the deep cerebellar nuclei is the main indicator of the gravity of the functional deficit caused by the degradation of that cell type. Namely the greater number of cells of a specific type and the closer proximity of those cells to the deep cerebellar nuclei, the greater deficit caused by the disease.
EXPERTISE: computational neuroscience

51. Restraint stress rapidly induces MAP kinase phosphatase-1 expression in the hypothalamic paraventricular nucleus.

C Osterlund, V Thompson, E Jarvis, RL Spencer.

Activity of CRH neurons in the hypothalamic paraventricular nucleus (PVN) is dependent upon tonic and phasic changes in corticosteroid levels and differing stress states. Stress induced activity of CRH neurons may be controlled in part through activation of the MAPK signaling cascade, including phosphorylation of MAPK. The activity of phospho-MAPK is governed by a tight feedback system involving the MAP kinase phosphatase (MKP) family. Although, MKP-1 expression is induced in various cell types by an array of extracellular stimuli, including corticosteroids, the possibility that MKP-1 expression in the PVN is dynamically regulated by corticosteroids or stress remains to be characterized. We examined the influence of restraint stress and corticosteroids on PVN MKP-1 mRNA expression (in situ hybridization) in young adult male Sprague-Dawley(SD) rats. In the absence of acute stress we observed no MKP-1 expression within the PVN. However, in rats challenged with 15 or 30min of restraint we observed a rapid up-regulation of PVN MKP-1 mRNA that was evident after 15min and pronounced after 30min of restraint. We also examined whether an acute increase in corticosterone (CORT) induces PVN MKP-1 mRNA, by treating rats with a 1hr or 3hr systemic CORT injection (2.5mg/kgi.p) before decapitation without intervening stress. Neither 1hr nor 3hr CORT treatment was sufficient to induce PVN MKP-1 mRNA levels. We also, examined whether the removal of endogenous corticosteroids by adrenalectomy (ADX) influences basal or stimulus-induced levels of MKP-1 mRNA. SD rats were ADX or shamed-ADX with a 5-day recovery before test day. ADX rats received CORT replacement in drinking water (25 µg/ml) that ended the night before testing. On test day all rats were challenged with 30min restraint and were then decapitated. Both sham and ADX rats showed MKP-1 expression after 30min restraint. Moreover, ADX rats exhibited a greater increase in PVN MKP-1 mRNA expression compared to sham rats. Thus the absence of tonic and acute CORT activity in ADX rats resulted in an increase in stress-induced PVN MKP-1 mRNA, implying that CORT may have suppressive effects on MKP-1 expression in the PVN. Stress-induced MKP-1 expression may play a critical role in mediating negative-feedback control of stress-induced MAP kinase cascades in the PVN.

52. Ligand dependent and independent activity of TAM family members in GnRH neuron development and reproductive function.

A Pierce[1], M Xu[1], B Bliesner[1], G Lemke[2], J Richards[3], S Tobet[4], and ME Wierman.[1]
[1]From the Division of Endocrinology, Diabetes and Metabolism at University of Denver at Denver and Health Sciences Center, Aurora, CO; [2]From The Molecular Neurobiology Laboratory at The Salk Institute, La Jolla, CA; [3]From the Department of Cell Biology at Baylor College of Medicine, Houston, TX; [4]From the Department of Biomedical Sciences at Colorado State University, Fort Collins, CO

AXL and TYRO3, members of the TYRO3, AXL, and MER (TAM) family of tyrosine kinase receptors, contribute to GnRH neuronal cell migration, survival and gene expression. AXL and TYRO3 are expressed in a variety of tissues, including the brain and ovary but not the vagina. Growth Arrest Specific gene 6 (Gas6) is a secreted protein that acts as ligand for TAM family members and has been shown to be involved in AXL- and TYRO3-mediated migration and survival as well as MER-mediated phagocytosis of apoptotic cells. Mice null for Axl and Tyro3 (double knockout or DKO mice) exhibit a selective loss of GnRH neurons associated with delayed puberty and irregular estrus cycles. Pituitary and ovary function appear normal, but ovariectomized DKO mice demonstrate an impaired ability to mount a sex steroid-induced LH surge, suggesting that a central defect of the hypothalamic/pituitary/gonadal axis is responsible for the reproductive phenotype. Interestingly, mice that are null for Gas6 also exhibit a decrease in the total GnRH population and delayed puberty, but estrus cyclicity is unaffected. Examination of vaginal histology suggests that the Gas6 phenotype is vaginal, not central, in origin and supports our hypothesis that AXL and TYRO3 may function in both ligand-dependent and ligand-independent mechanisms.

EXPERTISE: assessment of mouse female reproductive function

53. Restoration of the GnRH system in aging male transgenic animals paired with females.
JR Rochester, WCJ Chung, P-S Tsai.
From the Department of Integrative Physiology, University of Colorado, Boulder, CO.
Gonadotropin-releasing hormone (GnRH) neurons are required for the establishment and maintenance of fertility in all vertebrates. Previous studies have shown that there is little or no age-dependent decline in the number of GnRH neurons in normal rodents. However, we have shown that animals with target expression of a dominant negative FGF8 receptor in GnRH neurons (termed 'dnFGFR' animals) suffer a greater than 50% decrease in the GnRH system at approximately 300 days of age. Because approximately 10-20% of hypogonadotropic hypogonadal human patients harbor mutations leading to FGF signaling deficiency, we used a rodent model with similar FGF signaling deficiency to investigate if the postnatal loss of GnRH neurons can be restored by behavioral stimulation. We quantified GnRH neurons in aged male mice (approximately 550 days old) that had been housed with females and allowed to raise pups ("opposite-sex housed") or with other males ("same-sex housed"). Age-matched non-transgenic animals have a total of approximately 800 GnRH neurons regardless of whether they were opposite-sex housed or same-sex housed. As previously shown, same-sex housed dnFGFR mice had a >50% reduction in GnRH neurons compared to controls. Interestingly, housing dnFGFR males with females restored the number of their GnRH neurons by approximately 40%. We have shown, for the first time, that behavioral stimulation (i.e. opposite-sex pairing, mating, and/or rearing of young) rescues the decline in the aging GnRH system. While it is unclear what mechanisms are responsible for this rescue, these results provide compelling evidence that the neuroendocrine brain is a highly plastic and dynamic structure that responds robustly to environmental stimuli.
These results also suggest lifestyle changes may be sufficient for the restoration of failing reproductive function in aging individuals.
EXPERTISE: Immunocytochemistry, histology prep, sectioning, radioimmunoassay, DNA/mRNA isolation, tissue culture, behavior testing (birds).

54. Paraventricular Nucleus of the Hypothalamus: Novel Vascular Development and Relation to Adjacent Cell Types.
Melanie Schow, J. Gabriel Knoll, Qian Zhang, Stuart Tobet.
From the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO and College of Veterinary and Biomedical Sciences, Fort Collins CO
The vascular system is closely tied to brain development as well as function. The region of the paraventricular nucleus of the hypothalamus (PVN) is relatively unique in the central nervous system (CNS) for its extensive vascular supply that is easily distinguished from surrounding vessels by its dense "wing-shaped" pattern of vessels flanking the third ventricle. There is little known about the development, potential sex differences, or functional relevance of the unique vasculature of this region. The PVN itself is a heterogeneous nucleus with multiple neuronal cell types responsible for modulating different aspects of physiology. Two important populations of neurons are those that synthesize vasopressin (antidiuretic hormone), which functions to regulate blood pressure, and those that synthesize corticotrophin-releasing hormone (CRH), which plays a major role in stress responses. We are investigating the development of the PVN vasculature in relation to the surrounding cells and endocrine hormones produced in this region. We have observed the pattern formation in mice from embryonic day (E) 17 to adulthood using several visualization techniques. From these observations, it is apparent that multiple changes to the vasculature occur during development and that the PVN pattern does not fully develop until weaning (around postnatal day 19). Currently, we are using computer assisted image analysis based on brightfield microscopy with immunoreactive platelet endothelial cell adhesion molecule (PECAM) to measure the area of blood vessels in 50µm sections. From this measurement, we have identified a possible sex difference showing that the PVN area in females may be greater than in males. We are also using dual-fluorescence confocal microscopy in thicker sections

(250μm) to measure vascular volume and proximity of other cell types to blood vessels. In the longer term we plan to visualize the relationship between cells and vessels and their relative placement to each other in three dimensions to better understand the development of this uniquely vascularized region.
EXPERTISE: Immunocytochemistry, PCR, fluorescent and brightfield microscopy, perfusions and animal handling

55. Fear-potentiated startle induces c-Fos expression in serotonergic neurons selectively within the dorsal part of the mid-rostrocaudal dorsal raphe nucleus.
BM Spannuth, AK Evans, JL Lukkes, MW Hale, S Campeau, CA Lowry.
From the Department of Integrative Physiology and Center for Neuroscience, University of Colorado, Boulder, CO.
Midbrain serotonergic systems are known to be dysregulated in stress-related psychiatric disorders but the acute mechanisms leading to this dysregulation are not understood. Since such an understanding is a prerequisite to defining the mechanisms underlying dysregulation of serotonergic systems, knowledge of mechanisms underlying acute stress-induced activation of serotonergic neurons could play a vital role in the prevention of stress-related mental disease. The present study, aimed at understanding neural mechanisms underlying acute activation of serotonergic-systems by stress, used a fear-potentiated startle (FPS) paradigm since acoustic startle by itself has been shown to increase tryptophan hydroxylase activity and to increase extracellular serotonin in limbic forebrain sites, but not to result in the induction of the protein product of the immediate-early gene, c-fos, often used as a marker for cellular activation. Two experiments were performed in the present study, 1) an experiment confirming that footshock applied during training increases c-Fos expression in serotonergic neurons, and 2) an experiment investigating the effects of FPS on c-Fos expression in serotonergic neurons within the dorsal raphe (DR) and median raphe nuclei. In these experiments we demonstrate that 1) footshock increases c-Fos expression in serotonergic neurons throughout the DR and 2) the effects of FPS are mainly limited to the dorsal part of the mid-rostrocaudal dorsal raphe nucleus (DRD). These data are consistent with previous studies demonstrating that stress- and anxiety-related stimuli selectively increase c-Fos expression in the DRD. Identification of neural mechanisms underlying the regulation of this mesolimbocortical serotonergic system could have important implications for understanding the mechanisms underlying vulnerability to stress-related psychiatric disorders including depression and post-traumatic stress disorder. Supported by a UROP, BURST, and NSF REU grant to BMS and NIH R01MH086539 to CAL

56. Urocortin 2 activates a subpopulation of ventricle-projecting serotonergic neurons in the dorsal raphe nucleus.
CE Stamper, MW Hale, DR Staub, CA Lowry.
From the Department of Integrative Physiology and Center for Neuroscience, University of Colorado, Boulder, CO.
The neurotransmitter serotonin plays an important role in the regulation of anxiety states and physiological responses to aversive stimuli. Intracerebroventricular (i.c.v.) injection of the stress- and anxiety-related neuropeptide urocortin 2 (Ucn 2) increases c-Fos expression in serotonergic neurons in the dorsal part of the caudal dorsal raphe nucleus (dDRC). It has been shown that this region of the dorsal raphe nucleus contains a subset of serotonergic neurons that projects via the dorsal raphe periventricular tract to the subfornical organ, ependymal cells and the ventricular system. It is unclear if Ucn 2 injection is selectively activating this population of neurons. To determine if Ucn 2 activates ventricle-projecting serotonergic neurons in the midbrain raphe complex we made i.c.v. injections of the retrograde tracer Fluoro-gold into the lateral ventricle, followed 7 days later by i.c.v. injection of Ucn 2. Dual label brightfield immunohistochemistry replicated our previous finding that i.c.v. Ucn 2 increases c-Fos expression in serotonergic neurons in the dDRC. The dorsal part of the mid-rostrocaudal and caudal dorsal raphe nucleus, including the dorsal part of the dorsal raphe nucleus (DRD) at –8.18 mm, the DRC at –8.54 mm and the

dDRC at –9.16 mm Bregma was then analyzed using combined brightfield and immunofluorescence techniques. Approximately 40% of the ventricle-projecting neurons in the subdivisions of the dorsal raphe nucleus sampled were serotonergic. Intracerebroventricular injections of Ucn 2 increased c-Fos expression in non-ventricle-projecting serotonergic neurons in all of the sampled subdivisions and in ventricle-projecting serotonergic neurons in the DRC and dDRC at –8.54 mm and –9.16 mm Bregma respectively. Of the total population of ventricle-projecting serotonergic neurons, 20% expressed c-Fos following Ucn 2 injections. These data suggest that i.c.v. injection of the stress- and anxiety-related neuropeptide Ucn 2 activates a topographically organized and functionally distinct subpopulation of serotonergic neurons in the DRC. Supported by a UROP/HHMI Individual Grant to CES, a Wellcome Trust Research Fellowship to CAL (RCDF 068558/Z/02/Z) and NIH R01MH086539 to CAL.
EXPERTISE: immunohistochemistry, immunofluorescence

57. Exposure to an open-field in low-light and high-light conditions increases c-Fos expression in phenotypically distinct subpopulations of neurons in the rat basolateral amygdala.

AM Westerman, MW Hale, CA Lowry.
From the Department of Integrative Physiology and Center for Neuroscience, University of Colorado, Boulder, CO.

Anxiety states and anxiety-related behaviors appear to be regulated by a distributed and highly interconnected system of forebrain structures including the basolateral amygdala. We have previously reported that exposure to an open-field arena in both low-light and high-light conditions increases c-Fos expression in subdivisions of the basolateral amygdala including a marked increase in c-Fos expression in the anterior part of the basolateral amygdaloid nucleus (BLA) compared to either home cage or handled control groups. However, the neurochemical phenotype of the c-Fos-immunoreactive cell populations is not known. In this study, we investigated the effects of exposure to a novel open-field environment, with either low- or high-levels of illumination, on expression of the protein product of the immediate-early gene c-fos in populations of GABAergic interneurons expressing the calcium binding protein, parvalbumin (PV; as a marker of a subset of GABAergic interneurons expressing the serotonin 2A (5-HT2A) receptor), and glutamatergic neurons expressing calcium/calmodulin dependent protein kinase II (CaMKII; as a marker of glutamatergic basolateral amygdala projection neurons). Exposure to the open-field test in the low-light condition increased c-Fos expression in PV-immunoreactive neurons in the BLA, while exposure to the open-field arena in both low- and high-light conditions increased c-Fos expression in CaMKII-immunoreactive neurons in the BLA. These results suggest that exposure to mild anxiogenic stimuli activate phenotypically distinct neuronal populations in the BLA, and may provide a potential mechanism for the involvement of serotonin in the modulation of anxiety states and anxiety-related behavior.
Supported by a UROP/HHMI Individual Grant to AMW, a Wellcome Trust Research Fellowship to CAL (RCDF 068558/Z/02/Z) and NIH R01MH086539 to CAL.
EXPERTISE: Immunohistochemistry, mounting, anxiogenic stimuli

58. Presence of GnRH system in the mouse heart

H. Talbott, D. C. Skinner, Tao-Yiao John Wu.

Gonadotropin releasing hormone (GnRH) is most widely known for its role in reproduction. However, there is evidence for roles for GnRH outside of the HPG axis; including in the heart. In zebrafish GnRH has been implicated in proper heart development. Also, GnRH agonists used for the treatment of prostate cancer cause increased levels of cardiovascular disease in comparison to castration. The purpose of this study was to examine mouse heart tissue and confirm expression of GnRH, GnRH receptors, and EP24.15, a protease that degrades GnRH. We believe that the heart contains a GnRH system that is localized and independent from the HPG axis. Male mice were divided into two groups: non-treated mice and those treated with a chronic release implant containing deslorelin, a GnRH agonist. Reverse-transcriptase PCR was used to determine the

presence of GnRH and GnRH receptor mRNA in non-treated tissues. Western blots were used to detect EP24.15 in cellular fractions of plasma membrane (PM), nuclei (Nu), and cytoplasm (Cy) of normal and deslorelin treated mice were to detect EP24.15 using Western blots. Also, the activity levels of EP24.15 in the cellular fractions of normal and treated tissues were determined. In normal male mice hearts both GnRH and GnRH receptor mRNA were present. EP24.15 was present within the heart but after treatment with deslorelin the cellular distribution of EP24.15 changes, increasing in the PM and Nu, and decreasing in the Cy. The activity level of EP24.15 remains constant in all samples and in all fractions after deslorelin treatment. It is interesting to note that even though the distribution of EP24.15 appears to be changing EP24.15 activity levels seem to remain unaffected. These results indicate that all of the components needed for a complete GnRH system are present and systemic changes are occurring in the mouse heart after chronic deslorelin treatment. Support: University of Wyoming McNair Scholar's Program, University of Wyoming NASA Space Grant

59. Regulation of stress-induced gene expression in the hypothalamic paraventricular nucleus (PVN) and anterior pituitary by an acute PVN corticosterone microinjection.
MJ Weiser, J Highland, RL Spencer.
Dept. of Psych. and Neuroscience, University of Colorado, Boulder CO.
Endogenous glucocorticoid influence on the hormonal output of the hypothalamic-pituitary-adrenal axis (HPAA) is governed by its actions on a multitude of glucocorticoid-sensitive tissues and its differential effects within several distinct temporal domains. Corticosterone (CORT), the principal endogenous glucocorticoid in rats, can alter HPAA activity by acting at sites intrinsic to the axis (corticotropin-releasing hormone (CRH) neurons of the PVN and corticotrophs of the anterior pituitary(AP)), or by acting at brain sites extrinsic to the axis (e.g. hippocampus and bed nucleus of the stria terminalis). However, the relative contribution, and underlying molecular mechanisms, of intrinsic and extrinsic CORT actions on HPAA activity remain to be determined. In this study we examined the effect of an acute phasic rise in CORT at the level of the PVN on the expression of restraint-induced gene expression within the PVN and AP. On test day, adrenalectomized rats received an acute microinjection of CORT (10 ng, 0.5 ul) or vehicle into the PVN 1 hr prior to a 15 min restraint stress or home cage exposure. Restraint stress resulted in a significant induction of c-fos mRNA and CRH hnRNA within the PVN, and POMC hnRNA within the AP in vehicle injected animals. CORT microinjection blunted restraint-induced CRH hnRNA (PVN) and POMC hnRNA (AP), however had no effect on restraint-induced c-fos mRNA (PVN and AP) as compared to vehicle microinjection. These results indicate that CORT can act locally at the level of the PVN to inhibit phenotypic-specific immediate early gene (IEG) expression (CRH) in a short time frame (one hour), but not phenotypic-indiscriminant
IEG expression (c-fos). This suggests that an acute phasic increase in local CORT does not inhibit the stress-related neuronal input to the PVN or subsequent intracellular signal transduction that converges on c-fos gene induction. Whether this inhibitory effect of CORT on CRH gene activity reflects an intrinsic effect of CORT on CRH neuron function (e.g. direct glucocorticoid receptor action at the CRH gene promoter or altered excitation-gene expression coupling) or a trans-synaptic (peri-PVN) mechanism remains to be determined.

Sensory and Motor Systems

60. Mu-opioid receptor and its preferential endogenous agonist β-endorphin are expressed in the mouse retina.
J Angeln, J Mower, SK Gallagher and J Vigh.
From the Department of Biomedical Sciences, College of Veterinary and Biomedical Sciences, Colorado State University, Fort Collins, CO.
The endogenous opioid peptide, β-endorphin, is one of many diverse cleavage products of the large precursor protein proopiomelanocortin (POMC).The mu-opioid receptor (MOR) is a G protein-coupled receptor (GPCR) through which β-endorphin regulates many physiological functions, and is the target for most analgesic drugs. Expression of opioid peptides and their receptors in the

mammalian retina has not been studied in details and the existing sparse data is controversial. Methods: Using transgenic mouse models and immunohistochemical techniques we sought to characterize MOR and β-endorphin expression in the mouse retina through cell morphology, size, distribution, and colabeling studies with known retinal cell markers. Results: β-endorphin was shown to be expressed by a subpopulation of cholinergic (starburst) amacrine cells, of both orthotopic and displaced subtypes. Antibody directed against MORs stains neuronal somas and processes in the inner retina. MOR staining does not colocalize with POMC+ cholinergic amacrine cells. Instead, immunolabeling shows at least two distinct MOR+ cell-types in the inner nuclear layer (INL) and ganglion cell layer (GCL). Some MOR+ somas appear to be GABAergic, colocalize with the glutamic acid decarboxylases isoform GAD-67. No colocalization of MOR and the molecular marker for glycinergic amacrine cells (Gly-T1) was observed. Conclusion: Our findings point to a diverse population of putative amacrine cells and retinal ganglion cells that express the mu-opioid receptor and, through yet unidentified neuromodulatory pathways, may respond to the endogenous opioid peptide β-endorphin.
EXPERTISE: immunocytochemistry

61. Effect of carbon chain length, number and type on iron mediated lipid peroxidation of the brain, kidney, and liver.

WE Briner.
From the Dept. of Psychology, University of Nebraska at Kearney, Kearney, NE

Lipid peroxidation plays a role in a variety of pathophysiologic states. Metals often play a role in lipid peroxidation and iron is of particular interest because of the vital role it plays in a number of biological processes, as well as its abundance and ability to adopt the divalent of trivalent state. We have taken advantage of irons unique characteristics and attached carbon chains to both the 2+ and 3+ states that vary in structure and number of carbons. These molecules were then administered to mice to determine their LD50 and the amount of lipid peroxidation produced in the brain, liver and kidney. Iron with an attached pyridine chain produced significant lipid peroxidation in the brain but not so in the liver or kidney. The triple form of any of the synthesized molecules tended to produce greater lipid peroxidation. There was a general tendency for the more complex structure to produce greater lipid peroxidation in the liver but not the brain or kidney. The LD50 of the compounds increased with greater complexity. The LD50 was not related to the amount of lipid peroxidation in any organ. In conclusion pyridine-iron complexes appear to produce significant brain lipid peroxidation. Liver lipid peroxidation is directly related to the complexity of the structure. Kidney lipid peroxidation did not appear to be influenced by the structure of the iron-organic complexes.
EXPERTISE: Behavior, pharmacology, toxicology, heavy metals

62. Electrophysiology of Early Vision in Musca domestica.

K. Creaser[1], L. Benson[1], E. Tomberlin[2], S. Barrett1, C. H.G. Wright.[1]

The vision system of the common house fly, Musca domestica, has many interesting features including powerful early processing, a massively parallel configuration, and hyperacuity. Hyperacuity is the ability to detect movement at far greater resolution than predicted from photoreceptor spacing. We are investigating the role of constituent cells within the early vision processing of the fly through electrophysiological recording and modeling. Specifically, this research investigates the activity of the photoreceptors and monopolar cells L1, L2, and L4, in the lamina layer of fly's eye. These cells are thought to be key information processing locations. The monopolar cells' responses to varying stimulus frequency, intensity, and angle of approach were recorded and analyzed. Implications toward development of an analog sensor inspired by the vision system of the fly will also be provided.

63. Effects of pretreatment with clozapine on spatial memory of rats with lesioned dorsal hippocampi.

J Losacco, ME Basham.
From the Neuroscience Department at Regis University, Denver, CO.

The atypical antipsychotic clozapine improves spatial memory in rats with partial hippocampal lesions. However, the effectiveness of treatment with clozapine before brain injury is unknown. To assess the possibility that chronic pretreatment with clozapine might preserve spatial memory after a hippocampal lesion, we injected rats with either saline or clozapine for one month prior to partial hippocampal lesioning and then assessed spatial learning. Our initial results suggest that pretreatment with clozapine might moderate spatial memory deficits following partial hippocampal lesions. This finding has implications for the prevention and treatment of spatial memory deficits.

64. Cerebellum-directed aprotinin infusions inhibit plasmin activity but do not impair rat motor learning.

ME Basham, ML Skoch, EE Grange.
From the Neuroscience Program, Regis University, Denver, CO.

Tissue-type plasminogen activator (tPA) is involved in many examples of learning-related plasticity. For example, tPA mRNA is induced during the late phase of hippocampal LTP and tPA KO mice show impaired LTP whereas overexpression of tPA leads to enhanced LTP and improved performance in the Morris water maze. Similarly, tPA mRNA and protein are induced during cerebellar dependant motor tasks and pharmacological treatments that inhibit tPA activity impair rodent motor learning. In the periphery, tPA's primary role is the proteolytic conversion of plasminogen into the widely active protease plasmin. A similar role for tPA has been proposed in the cerebellum where tPA could facilitate synaptic remodeling through activation of the plasminogen/plasmin system. To test this hypothesis, we trained rats in a cerebellar-dependent motor task and blocked either tPA or plasmin activity during motor training. We report that inhibiting tPA activity through cerebellum-directed infusions of PAI-1 impairs motor learning, whereas inhibiting plasmin activity through cerebellum-directed infusions of aprotinin does not. These results suggest that tPA facilitates motor learning in rats through pathways that are independent of the plasminogen/plasmin system.