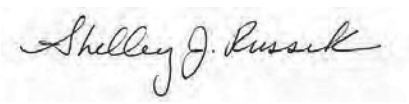
Welcome to the Twentieth Annual Henry I. Russek
Student Achievement Day!

How could twenty years have passed already when it feels like only yesterday we graduated our first class of award winners! There are so many new faces of dedicated individuals that will move us forward into the next exciting years and many dear friends that we have lost along the way whose impact on us personally and on the BU community will never be replaced. No rhyme or reason to the incessant rhythm of life and renewal; except here, where it beats with a single purpose as educators and mentors.

I am so proud of the members of our Division Awards Committee and the Program that they have helped me build. Some of these unique individuals have been with me from the first day and it is a true testament to their character that they have never thought twice about giving their time when it can benefit the welfare of a student. 2014 team: Drs. Marlene Oscar-Berman, Barbara Slack, Barbara Nikolajczyk, Jennie Luebke, Shoumita Dasgupta, Barbara Schreiber, Olga Gursky, Matt Jones, and Yuki Mochida.

We also thank all our student volunteers and the faculty in each department who had to choose between excellent applicants while putting up with my persistence regarding community service. Finally, with sincere appreciation: Sara Johnson (Pharmacology), Wanda Roberts (Pharmacology), Dr. Theresa Davies in GMS, Daniel Madigan in Ed Media, Jerry Lavoie (GMS), Kayleigh Klegraefe (GMS), Sabita Bandyopadhyay (Russek lab), Dr. David Farb, Mrs. Elayne Russek and the generous support of the Russek Foundation.

Have fun!



Excerpt from the Russek Lecture 1992 Journal of the American College of Cardiology (JACC) Must Cardiology Lose its Heart?

Delivered by Richard Gorlin, MD, FACC
New York, New York

As the Henry I. Russek Lecturer, I am mindful of the special honor of the invitation because Henry I. Russek was a personal friend as well as the paradigm of the medical humanitarian.

A Man of Science

I first met Henry back in the late 1950's while we were both interested in the actions of the nitrites on the ischemic heart. He believed in the widespread effects of the nitrite compounds in relieving angina pectoris and published some of the earliest reports showing an action of nitrites on both the exercise electrocardiogram and the inception of angina in patients with symptomatic coronary heart disease.

Henry I. Russek had a remarkable scientific prescience. As early as 1968 he described the synergistic effects of beta-adrenergic blocking agents and nitrites when given together. Moreover, in an era long before clinical trials, randomization, blinding and statistical analysis, he was one of the first to suggest that anticoagulant agents played a selective rather than a general role in the treatment of acute myocardial infarction. Without the biostatistical and study design tools we use today, he was able to determine that it was the patient at high risk for thrombosis who deserved the benefits and who could risk the hazards of anticoagulation. He identified the patients at risk as those with venous disease, arterial disease or congestive heart failure. Later in my own work, I cited this 1954 paper when discussing coronary heart disease.

A Man of Medicine

Perhaps more pertinent to this Henry I. Russek Lecture is what Henry was like as a man and physician. In the encomia for Henry provided by his family and by the College, one statement emerges repeatedly: he was a true physician-healer. Henry has been described as having a mind open to new science and new ideas. He was considered to be a student as well as educator and communicator. With his patients he was not only an astute diagnostician but a compassionate and active listener. Those who knew him well report that he knew how to heal the healer as well as the patient, and that he continually preached that humanism begins with humanizing the physician.

JACC Vol. 19, No.7
June 1992:635-640

Our Scientists

David D. Ginty, PhD

Investigator / 2000–Present

Scientific Discipline

Developmental Biology, Neuroscience

Related Links

The Ginty Lab

Host Institution

Harvard Medical School

Current Position

Dr. Ginty is also a professor of neurobiology at Harvard Medical School.

Education

- BSc, biology, Mount Saint Marys College
- PhD, physiology, East Carolina
- University School of Medicine

Awards

- Pew Scholars Award
- Klingenstein Foundation Award in Neuroscience
- Jacob Javits Neuroscience Investigator Award, National Institutes of Health

Show More

Current Research

Organization, Function, and Development of the Sensory Neurons of Touch

David Ginty's research addresses the function, organization, and mechanisms of assembly of peripheral nervous system and spinal cord circuits that underlie the sense of touch.

Read more ›

Biography

David Ginty tells the graduate students and postdocs in his lab to be fearless about learning and using new approaches. “You have to do whatever it takes to answer outstanding questions,” he says. “That means trying a new technique or coming up with one if it doesn’t already exist.” Ginty has followed this strategy throughout his career—grabbing molecular biology tools to delve into the processes responsible for laying out the complex networks of cells and connections that make up the vertebrate nervous system.

Ginty started working in this research field as a postdoc in John Wagner’s laboratory at the Dana-Farber Cancer

Institute in Boston, studying the mechanism of action of nerve growth factor (NGF). This protein was known to be critical to the survival of sympathetic neurons, a family of neurons originating from spinal ganglia—masses of nerve tissue near the spinal cord that contain the cell bodies of neurons—and reaching out to target organs such as blood vessels, the heart, and glands.

When Wagner announced he was moving to Cornell University in New York City, Ginty had to choose whether to follow or look for another position. “I agonized over the decision because my project was going well and I thought that if I switched labs I would lose momentum,” he says. But his wife had recently given birth to their first son and they thought Boston would be an easier place to raise a family. So, Ginty moved across the street to the laboratory of Michael Greenberg at Harvard Medical School.

That choice turned out to be a good one. In Greenberg’s lab, Ginty demonstrated that when NGF binds to its receptor on a nerve cell it “turns on” the activity of a molecule called CREB (cAMP response element binding). In the cell’s nucleus, CREB, a transcription factor, regulates the expression of a large cohort of genes that may control the growth, differentiation, and survival of neurons.

But to study how NGF regulates CREB, Ginty needed a way to easily measure whether CREB was phosphorylated—in other words, in its active form—or not. In a technical feat, Ginty developed the first antibody that specifically recognizes the phosphorylated form of CREB. “Once we had the antibody, my experiments became 1,000 times easier,” he says.

After establishing his own lab at the Johns Hopkins University School of Medicine, Ginty used his antibody in combination with a variety of technologies to ask how NGF, a signaling molecule that binds to a receptor located on the distal part of a nerve projection, or axon, could regulate CREB and other nuclear proteins located in the neuron’s cell body at the opposite end. Ginty’s lab discovered that when NGF binds its receptor, the complex is incorporated in a membrane-bound compartment called an endosome. Inside the endosome, NGF travels through the axon to the cell body, a meter-long journey for some human nerve cells, to activate molecular events that occur in the cell’s nucleus, including gene transcription.

Eventually Ginty’s quest to add more pieces to this puzzle brought him to studies in mice. In collaboration with HHMI investigator Alex Kolodkin, who had started his own lab at Hopkins at around the same time, Ginty began to study the function of another nerve growth signal called semaphorin. One of the first questions they asked was, “What is the receptor for this molecule?” Through a series of experiments they determined that a protein called neuropilin is the receptor for one type of semaphorin called Sema3A; another related protein called neuropilin-2 is a receptor for other semaphorins. They then engineered “knockout” mice lacking one or the other neuropilin genes, demonstrating that both molecules are required for semaphorin function in vivo. “That question is what first got us into mouse genetics,” says Ginty. “And I have not looked back since.”

For the past 10 years, Ginty has been taking advantage of sophisticated mouse genetic approaches to identify the key molecular events that underlie the growth and survival of neurons in the peripheral nervous system. One of his recent discoveries is that sympathetic neurons compete for their survival through a series of feedback mechanisms. As axons extend toward their targets, they are only modestly responsive to NGF and other survival signals produced by target tissues. But as they innervate their target, these neurons’ responses to NGF become amplified through processes that require the transcription of several genes, resulting in large differences among neurons. The neurons that are more responsive to NGF become stronger and “punish” their neighbors, by producing signals that harm them. As a result, the stronger neurons survive and the others perish. That is one way the organism ensures that the right number of nerve cells end up innervating the intended target.

“I love how powerful the molecular-genetic approach has been to understand how neurons grow, extend axons into target fields, mature, and survive,” says Ginty. “It has been fascinating on so many levels.” And as Ginty follows the paths of his neurons, he is constantly being taken to new research areas. “We have recently identified a subset of sensory neurons that send their axons to the skin to respond to touch,” he says. “There are so many questions we now want to ask.”

When Ginty is not chasing neurons he oversees the graduate program in neuroscience at Johns Hopkins, imparting his excitement about science to students. “I love working with students,” he says. “When I was a postdoc my heart was in doing experiments and in using my hands to answer questions. I thought I would have to keep doing that to enjoy science. But after I set up my own lab I realized that it is even more satisfying when a student or a postdoc in the lab has a breakthrough. There is nothing better in the world.

In November 2013, Dr. Ginty's lab moved from the Johns Hopkins University to Harvard Medical School.

Student Achievement Day 2014

Program of Events:

Coffee and pastries available at 8:30 a.m.
Please pick up badges and abstract booklet at the front of the Heibert lounge from our student hosts.
Please put up your poster before 9:00 a.m.

9:00-9:30 a.m.
Welcoming addresses by Dr. Shelley J. Russek (Vice-President, Russek Foundation, BU Professor of Pharmacology), Dr. Linda Hyman (Associate Provost, Division of Graduate Medical Sciences, Professor of Microbiology), and current President of the Graduate Medical Sciences Student Organization.

9:30-10:45 a.m.
Henry I. Russek Keynote Lecture
"The Sensory Neurons of Touch" by Dr. David Ginty, Professor of Neurobiology, HHMI Investigator, Harvard Medical School.

10:45 a.m.-1:00 p.m.
Viewing of posters presented by graduate students enrolled in the Division of Graduate Medical Sciences (Buffet luncheon available at 11:30 a.m.)

11:30 a.m.-1:00 p.m.
Award winners (First, Second, and Third) please get your lunch and bring to the student lounge (14th floor L-Bldg). You will be having lunch with our Keynote Speaker & Visiting Professor Dr. David Ginty.

1:00-3:45 p.m.
Slide presentations by the 2014 Henry I. Russek Student Achievement First Prize Recipients. (Each presentation is 10 min. with an additional 5 min. for questions. Student moderators will host the event.)

4:00 p.m.
Award presentations by Shelley J. Russek, Russek Foundation. Photo shoot of our wonderful award winners!

Oral Presentations

1:00-1:15 p.m.
Caitlin Leibowitz:
ACTIVATED PROTEIN C RELIEVES ER STRESS BUT NOT MORTALITY IN MOUSE KIDNEY INJURY.
(Department of Pathology, Advisor: D. Stearns-Kurosawa)

1:15-1:30 p.m.
Hila Milo Rasouly:
LOSS OF ZEB2 CAUSES GLOMERULOCYSTIC KIDNEY DISEASE IN MICE.
(Program in Genetics & Genomics, Advisor: W. Lu)

1:30-1:45 p.m.
Kristie Hilliard:
THE LUNG-LIVER AXIS FACILITATES INNATE IMMUNITY AND SURVIVAL DURING PNEUMONIA.
(Department of Microbiology, Advisor: L. Quinton)

1:45-2:00 p.m.
Kathleen Tumelty:
MECHANISMS OF AORTIC CARBOXYPEPTIDASE-LIKE PROTEIN REGULATION OF THE FIBROBLAST TO MYOFIBROBLAST TRANSITION.
(Department of Biochemistry, Advisor: M. Layne)

2:00-2:15 p.m.
Tara Vanderweyde:
REGULATED PROTEIN AGGREGATION OF TIA1 INCREASES TAU AGGREGATION AND NEUROTOXICITY IN VITRO.
(Department of Pharmacology & Experimental Therapeutics, Advisor: B. Wolozin)

2:15-2:30 p.m.

Anna Eisenstein:

AN ADENOSINE RECEPTOR-KRUPPEL-LIKE FACTOR 4 AXIS INHIBITS ADIPOGENESIS.

(Program in Molecular Medicine, K. Ravid)

2:30-2:45 p.m.

Samantha Michalka:

DYNAMIC RECRUITMENT OF HUMAN FRONTAL LOBE NETWORKS FOR TEMPORAL AND SPATIAL PROCESSING.

(Graduate Program for Neuroscience, Advisor: D. Somers)

2:45-3:00 p.m.

Lev Vaisman:

DIFFERENTIAL BLINKING RATES AS A FUNCTION OF HANDEDNESS: SERENDIPITOUS FINDINGS FROM A STUDY OF THE EVOKED P300 POTENTIALS RECORDINGS.

(Department of Anatomy and Neurobiology, Advisor: P. Bergethon & M. Moss)

3:00-3:15 p.m.

Stacy Andersen:

EPISODIC MEMORY AND EXECUTIVE FUNCTION IN FAMILIAL LONGEVITY.

(Program in Behavioral Neuroscience, Advisor: T. Perls)

3:15-3:30 p.m.

Anastasia Karabina:

THE EFFECT OF MYOSIN REGULATORY LIGHT CHAIN PHOSPHORYLATION ON B-MYOSIN MECHANICS IN HEALTH AND DISEASE.

(Department of Physiology & Biophysics, Advisor: J. Moore)

3:30-3:45 p.m.

Debora Heller:

THE EFFECT OF SERUM ON IN VIVO EARLY MICROBIAL COLONIZATION OF TOOTH ENAMEL.

(Program in Oral Biology, Advisor: F. Oppenheim)

Recipients of the Henry I. Russek Student Achievement Awards 2014

First Prize

Stacy Andersen
Program in Behavioral Neuroscience
Advisor: T. Perls

Anna Eisenstein
Program in Molecular Medicine
Advisor: K. Ravid

Debora Heller
Program in Oral Biology
Advisor: F. Oppenheim & E. Helmerhorst

Kristie Hilliard
Department of Microbiology
Advisor: L. Quinton

Anastasia Karabina
Department of Physiology and Biophysics
Advisor: J. Moore

Caitlin Leibowitz
Department of Pathology
Advisor: D. J.Stearns-Kurosawa

Samantha Michalka
Graduate Program for Neuroscience
Advisor: D.Somers

Hila Milo Rasouly
Program in Genetics and Genomics
Advisor: W. Lu

Kathleen Tumelty
Department of Biochemistry
Advisor: M. Layne

Lev Vaisman
Department of Anatomy and Neurobiology
Advisor: P. Bergethon & M. Moss

Tara Vanderwedye
Department of Pharmacology & Experimental Therapeutics
Advisor: B. Wolozin

Second Prize

Madhurima Das
Department of Physiology & Biophysics
Advisor: O. Gursky

Kelsey Derricks
Program in Molecular Medicine
Advisor: M. Nugent

Samantha Hiemer
Department of Biochemistry
Advisor: R. Varelas

Natasha Khatri
Department of Pharmacology & Experimental Therapeutics
Advisor: H. Man

Philip Montenigro
Department of Anatomy and Neurobiology
Advisor: R. Stern

Nicole Stauffer
Department of Pathology
Advisor: J. P. Mizgerd

Mariepierre Surpris
Graduate Program for Neuroscience
Advisor: J. F. Chen

Third Prize

Carly Garrison
Program in Genetics and Genomics
Advisor: A. Spira

Anna Pisarek-Horowitz
Program in Molecular Medicine
Advisor: W. Lu

Department of Anatomy & Neurobiology

NOTE: Prize winners are noted in the following list of Abstracts by:
**1st Prize, **2nd Prize, ***3rd Prize*
The accompanying number indicates each abstract's poster board.

Participants

Jonathan Dashkoff (52)
Sherri Eldridge (16)
Danielle Farrar (55)
Bruno Frustace (15)
Abenet Ghebremichael (47)
Joseph Goodliffe (36)
Nadine Heyworth (32)
*Philip Montenigro (23**)*
Julie Stamm (70)
Alex Stankiewicz (3)
Lev Vaisman (*)
Wang Xiyue (54)
Chen Yuan Yang (2)
Lauren Zajac (38)

*

DIFFERENTIAL BLINKING RATES AS A FUNCTION OF HANDEDNESS: SERENDIPITOUS FINDINGS FROM A STUDY OF THE EVOKED P300 POTENTIALS RECORDINGS

Vaisman, Lev; Bergethon, Peter R.; Moss, Mark B.
Department of Anatomy and Neurobiology.

Electroencephalography (EEG) is a technique of recording the electrical activity of the brain using scalp electrodes. EEG signal is susceptible to eye blink artifacts. Eye blinking is a protective opening and closing of the eye. The rate of blinking is controlled by the caudate nucleus and dopaminergic pathways in the brain. In this study, a number of different subject-specific and experimental factors were analyzed with a purpose of seeking implementable experimental design modifications that would allow reducing the amount of blink artifacts during the EEG recordings of cognitive experiments. Significantly lower blinking rates in left-handed people compared to right-handed ones was found during the oddball sequences recordings (p-value = 1.4e-7). This finding, surprisingly previously unreported in literature, was also robust when study participants were grouped by gender (p-value for males = 7.3e-7 and p-value for females = 9.7e-3). Based on this finding, an epidemiologic study was performed using the Michael J. Fox Foundation's PPMI database for the study of Parkinson's disease (PD) to investigate, whether there was a relationship between person's handedness and the side of the body, in which he / she develops dominant PD symptoms. No significant relationship was found (odds ratio 95% confidence interval 0.59-1.83). For the future work, it may be useful to attempt replicating blinking rate variation with handedness in a large sample of study participants. If confirmed, this variation will be an important factor in the design of the dopaminergic medications trials, in which blinking rates are used to quantify the medications effects.

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RESTRICTED EXPRESSION OF TRANSGENE TO ASTROCYTES IN THE CENTRAL NERVOUS SYSTEM FOLLOWING SYSTEMIC INJECTION WITH A NOVEL SELF-COMPLEMENTARY AAV9 VECTOR

Dashkoff Jonathan; Hudry, Eloise; Fan, Zhanyun; Maguire, Casey; Takeda, Shuko; Tannous, Bakos A.; Hyman, Bradley T.

Department of Anatomy and Neurobiology

Alzheimer's disease (AD) is a devastating neurodegenerative disease for which there is currently no cure. Recently, we demonstrated that expression of the *epsilon2* allele of human apolipoprotein E (*APOE*) by intraventricular injection of adeno-associated virus serotype 4 (AAV4) can abrogate pathological processes in transgenic mouse models of AD (Hurdy, Dashkoff, *et al,* 2013). To further enhance the safety and efficacy of this therapeutic approach, we engineered a novel self-complementary AAV9 vector that expresses transgene under the control of a fragment of the full-length glial fibrillary acidic protein (GFAP) promoter. Intravenous delivery of this viral vector encoding green fluorescent protein led to robust transduction of astrocytes throughout the CNS in the absence of neuronal transduction that persisted for two months after injection. The virus transduced activated and non-activated astrocytes as determined by immunohistochemical staining using antibodies against GFAP and glutamine synthetase, respectively. Quantitative stereological assessment demonstrated that transduction with AAV9 is not associated with reactive gliosis. This data suggest the potential of AAV9 for the treatment of AD and future studies aimed at using this system to successfully drive expression of *APOE epsilon2* in astrocytes are ongoing.

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SENSORY INNERVATION IN THE SKIN OF HUMPBACK WHALES (*Megaptera novaeangliae*)

Sherri Eldridge; Mortazavi, F.; Rosene, D.L.

Marine environmental signals provide critical information to cetaceans (whales, dolphins and porpoise) for finding prey, navigating migratory routes, assessing dive depth pressures, and interpreting and triangulating communication calls. While mechanosensory processes of the auditory systems are known, somatosensory contributions from innervation in marine mammal glabrous skin remain unknown. Ears are finely tuned to frequency and amplitude modulation, while skin receptors respond to temperature, chemoreception, nociception, tactile stimuli, pressure changes, and vibrational waves. For example, the body of whales may provide a long sensory array for triangulation of long wavelength, low-frequency conspecific call signals. Here, we identify anatomical features of cutaneous innervation in whale skin. Biopsies collected from the flanks of humpback whales (*Megaptera novaeangliae*) (NMFS Permit #15240) were fixed, frozen and sectioned for immunohistochemistry. Techniques were optimized to visualize antibodies specific for highly conserved molecules marking axons, low-threshold mechanoreceptors (LTMRs) and neural supporting cells known to exist in the thick skin of terrestrial mammals, such as the footpads of mice. Neural fiber types, axonal endings, structural features, and locations were identified. Whale skin has a highly vascularized subepidermal neural plexus containing multiple fiber types (some with LTMRs), extensive branches into lengthy dermal papillae, and fine fibers terminating in the epidermal stratum basale. Compared to terrestrial mammals, whale skin showed an enlarged diameter of fibers and insulating support cells, a lack of encapsulated sensory end-organs, and novel organization of dendrites in the fascicles. Variations from the primitive terrestrial condition are likely derived adaptations of mammalian skin to an aquatic environment.

55

FUNCTIONAL CONNECTIVITY DIFFERENCES IN MCI PATIENTS WITH GOOD VERSUS DIMINISHED EXECUTIVE CAPABILITIES

Farrar, Danielle; Budson, Andrew; Moss, Mark; Killiany, Ron

Mild Cognitive Impairment (MCI) is a condition that affects 10-20% of adults 65 and older and often progresses to Alzheimer's disease. Most imaging investigations into the cognitive deficits associated with MCI have focused on memory function in patients with so-called MCI of the amnestic type. However, deficits in the executive function realm, such as impaired decision making ability, can be more devastating to an individual's well-being than memory deficits. Using data from the Alzheimer's Disease Neuroimaging Initiative, we examined the resting state network connectivity of 30 individuals with an MCI-amnestic diagnosis. 15 of the subjects were classified as "high executive functioning individuals" and 16 were classified as "low executive functioning" individuals, based on neuropsychological test performance. Regions of interest (ROIs) were identified based on MRI anatomical scans, and a Pearson correlation of all ROI pairs determined functionally connected regions of the resting state fMRI scans obtained from these individuals. Using the Network Based Statistic (NBS) algorithm on the populations to correct for multiple compairsons, we found a significant decrease in connectivity between the frontal-parietal and temporal network in the low executive function performers. This findings offer insights into how MCI impacts the brain and may provide the basis for future interventions.

THE PHYSIOLOGIC CORRELATES OF LEARNING IN THE CLASSROOM ENVIRONMENT

Frustace, Bruno; Budson, Andrew; Flannery, Sean; Ghebremichael, Abenet; Iyer, Alex; Tat, Michael; Zumwalt, Ann

Anatomy & Neurobiology Department

This study served to further investigate learning and memory, and to offer a potential tool to support educational interventions. This was accomplished by an investigation of the physiologic changes in the brain that occurred while students learned medical anatomy. A group of 29 students taking the Gross Anatomy course at Boston University School of Medicine participated in the study. Testing occurred in two sessions: prior to the course and at the completion of the course. For each session, scalp EEG was recorded while participants were shown 176 anatomical terms (132 relevant to the course and 44 obscure) and asked to respond with "Can Define", "Familiar", or "Don't Know". Behavioral results indicated a positive correlation between participants' course grades and performance on the experimental tasks. EEG results were analyzed for event-related potential (ERP) components related to two memory components: familiarity and recollection. For Don't Know responses, a stronger early frontal, late parietal, and late frontal effect occurred for terms of Session 1 compared to Session 2. Analysis of Session 2 data indicated increased activity of the early frontal, late parietal, and late frontal effects for Can Define responses only. Session 2 Can Define responses elicited a stronger early frontal ERP, occurring between 300 and 500 milliseconds yet, the most post-retrieval processing and monitoring appeared for Can Define terms of Session 2. Ultimately, we focused on investigating two points: 1) the effect of classroom learning on memory, and 2) the examination of ERPs as a tool to guide education interventions. Specifically, ERPs would potentially indicate markers to predict whether students would retain materials long before behavioral measures indicate these results. This has potential to determine whether long-lasting or transient learning will occur; as well as the potential to support early intervention strategies for not just students, but also individuals with learning disabilities or memory impairments.

GAZE PATTERNS OF ANATOMY STUDENTS THROUGH CLASSROOM LEARNING AND FAMILIARIZATION

Ghebremichael, Abenet
Anatomy & Neurobiology

This study aims to identify the gaze patterns of medical students as they correlate with learning and familiarization through the length of a course. The gaze patterns of medical gross anatomy students (n=31) were documented as they identified anatomical structures on a computer screen. Each student took the test before the start of the Human Gross Anatomy course, and was randomly assigned to a group (A, B, or C) that would take it after one of three course section exams, Back and Limbs, Thorax Abdomen Pelvis, and Head and Neck, respectively. Their gaze patterns were expected to change as they become more familiar with the course material, particularly with respect to cognitively salient Areas of Interest (cAOIs) that are relevant to identifying the tagged structure. We predict that unfamiliar students will demonstrate more saccadic movements, shorter fixation times on cognitively salient AOIs, and longer fixation times on visually salient AOIs when compared to experienced students. Predictions that saccade frequency would decrease with familiarity and that fixation time in visually salient AOIs would decrease were not upheld. There appears to be a decrease in fixation time on the area surrounding the AOIs (White Space) for groups of subjects familiar with the material. This is found to be a statistically significant decrease in Group B's Back and Limbs ($p = 0.038$) and Thorax Abdomen Pelvis ($p = 0.000$) sections as well as Group C's Back and Limbs section ($p = 0.013$). This decrease in fixation time on the White Space is due to an increase in fixation time on cognitively salient AOIs with the only statistically significant increase occurring in Group C's Thorax Abdomen Pelvis section.

NEUROPATHOLOGIES OF THE TS1YEY MOUSE MODEL OF DOWN SYNDROME

Goodliffe, Joseph; Haydar, Tarik
Anatomy and Neurobiology

Down syndrome (DS) is the leading genetic cause of intellectual disability. Mouse models of DS have been engineered to study the etiology of Hsa21-homolog triplication and the Ts65Dn model has been shown to exhibit deficits in neurogenesis, cortical hypocellularity, and an imbalance of excitatory/inhibitory neuronal populations. Ts1YEY is a novel mouse model in which the entire Hsa21-homologous region of mouse chromosome 16 (Mmu16) has been triplicated. To date, few studies have investigated the cellular and morphological phenotypes of the pre- and post-natal Ts1YEY. In this study, we investigated the effects of segmental trisomy on pre- and postnatal brain development. Ts1YEY embryos show a reduction in overall brain and embryo size at specific stages of development. The proliferative neural stem cell population and mitotic events of the ventral and dorsal germinal zones are similar in Ts1YEY and euploid littermates while the intermediate progenitor population is larger in Ts1YEY neocortex. In early postnatal life, Ts1YEY cortex exhibits hypocellularity in both excitatory and inhibitory neuronal populations which shifts the cortical excitatory/inhibitory ratio. The abnormalities of the Ts1YEY do not fully mirror those reported in the Ts65Dn. Observed differences between models may arise from the size of the triplicated region, epigenetic modifications due to chromosome engineering, or Ts1YEY-specific genes acting in a compensatory manner. In order to substantiate the use of the Ts1YEY model the mechanism underlying these differences must be elucidated.

Work supported by NIH, NICHD/NIMH, RO1HD05780

AGE-RELATED ALTERATIONS OF OLIGODENDROGLIA IN WHITE MATTER OF THE NON-HUMAN PRIMATE

Heyworth, Nadine; Carmichael, Alana; Rosene, Doug

Anatomy and Neurobiology

Adult neurogenesis occurs in specific regions of the brain including the Subgranular Zone of the hippocampal dentate gyrus and the Subventricular Zone of the lateral ventricle. These new neurons are known to be important for specific cognitive tasks such as pattern separation. However, these neurons do not appear to contribute to the age-related decline in cognitive performance on other hippocampal based tasks such as Delayed Non-Matched to Sample or Delayed Recognition Span Task. Alternative factors in aging, such as white matter integrity, have been shown to correlate with cognitive decline. Evidence of re-myelination has been documented in the adult brain, but myelin profiles in the aged brain exhibit pathologic changes including split sheaths. It is unknown if age-related changes in the myelin result from alterations or dysfunction in oligodendrocytes, the myelinating cells of the central nervous system. While adult neurogenesis is restricted to two neurogenic regions, gliogenesis is ongoing throughout the adult brain. This study used immunohistochemistry to quantify age-related changes in oligodendrocyte progenitor cells (OPCs) and mature oligodendrocytes in cognitively relevant regions of the brain. Preliminary results from this study indicate that levels of OPCs and mature oligodendrocytes are not altered with age in the fornix, temporal lobe white matter, temporal lobe gray matter, superior longitudinal fasciculus or genu of the corpus callosum. Interestingly, there was an age-related increase in mature oligodendrocytes in the cingulum bundle. Further analysis will be conducted to examine the role of oligodendrocyte proliferation and survival in the context of remyelination and cognitive function.

23**

SUBCONCUSSIVE IMPACTS AND THE RISK OF LATER-LIFE DEPRESSION AND COGNITIVE IMPAIRMENT IN FORMER HIGH SCHOOL AND COLLEGIATE FOOTBALL PLAYERS

Montenigro, Philip; Baugh, Christine; Cantu, Robert; Daneshvar, Daniel; Martin, Brett; McClean, Michael; McKee, Ann; Nowinski, Christopher; Seichepine, Daniel; Stern, Robert; Tripodis, Yorghos

Anatomy and Neurobiology

The relation between concussions and clinical impairments is recognized. In contrast, the acute and long-term clinical significance of asymptomatic, subconcussive impacts (SCI) remains unclear.[1] In this study, we tested the hypothesis that greater SCI exposure increases an athlete's risk of developing later-life impairment. We developed the Life-Load Index (LLI), which is a summary estimate of an athlete's total impact exposure, based on variables such as the number of years played, the position(s) played, and previous findings of head impact g force for each position.[2,3] Participants included 39 former high school and 74 former college football players. Statistical modeling indicated a quadratic relationship between SCI exposure and later-life impairment. A logistic regression was conducted with the LLI as the predictor and clinical impairment in behavior (BRIEF-A BRI T-score >65), cognition (BRIEF-A MI T-score >65), and mood (CES-D depression cut-off score >22) as dependent outcomes.[4,5] All regressions were adjusted for # of concussions, age at first exposure to football, education, and age. Our results show that for every 1000 estimated impacts, the odds of reporting severe depression increased by 25% (CES-D; p=0.028), and the odds of reporting clinical cognitive impairment increased by 19% (MI; p=0.0691). Behavior was not significantly related to exposure. This is the first study to report a dose-response relation between subconcussive accumulated exposure and later-life impairment in former amateur athletes. Our preliminary findings have important implications for public health but require validation.

References

1. Bailes, JE., Petraglia, AL., Omalu, BI., Nauman, E., and Talavage, T. "Role of subconcussion in repetitive mild traumatic brain injury: A review." Journal of neurosurgery, 2013.
2. Beck, M., Mulkey, LA., and Barnwell, TO. "Model Validation for Exposure Assessments." Athens, Georgia: 1994.
3. The United States Environmental Protection Agency (EPA) "Guidance on the Development, Evaluation, and Application of Environmental Models." March 2009 www.epa.gov/crem
4. Roth, RM., Isquith, PK., and Gioia, GA. "BRIEF-A: Behavior Rating Inventory of Executive Function--adult Version: Professional Manual." Psychological Assessment Resources, 2005.
5. Radloff, LS. "The CES-D scale a self-report depression scale for research in the general population." Applied psychological measurement, 1977.

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RELATIONSHIP BETWEEN PLAYING TACKLE FOOTBALL PRIOR TO AGE 12 AND LATER-LIFE MOOD, BEHAVIOR, AND EXECUTIVE FUNCTIONING

Stamm, Julie; Au, Rhoda; Baugh, Christine; Bourlas, Alexandra; Breaud, Alan; Cantu, Robert; Daneshvar, Daniel; Gioia, Gerard; Martin, Brett; McClean, Michael; McKee, Ann; Nowinski, Christopher; Ozonoff, Al; Riley, David; Robbins, Clifford; Stern, Robert; Tripodis, Yorghos

Anatomy and Neurobiology

With millions of youth athletes participating in contact sports, youth sports-related brain trauma is an important public health issue. Research suggests repetitive brain trauma may have long-term neurological consequences in some athletes. However, the relationship between age of first exposure (AFE) to this trauma and later-life impairment is unclear. Our objective was to examine the relationship between AFE to tackle football and later-life mood, behavior, and executive function. We hypothesized that subjects who started playing football before age 12 would demonstrate greater later-life impairments than subjects who began playing at age 12 or older. Subjects included 92 male former football players selected from our LEGEND study and divided into two groups based on AFE to football: AFE <12 and AFE ≥12. Outcome measures included the Apathy Evaluation Scale (AES), Center for Epidemiologic Studies Depression Scale (CES-D), and Behavior Rating Inventory of Executive Function-Adult Version (BRIEF-A). The AFE <12 group scored significantly worse on the AES (p=0.004) and BRIEF-A Global Executive Composite (GEC; p=0.003) and Behavior Regulation Index (BRI; p=0.001). A significantly greater proportion of subjects in the AFE <12 group had clinically meaningful scores on the CES-D (≥16; p=.03) and GEC (≥65; p=.03). Bootstrapped adjusted odds ratios indicated an approximately three fold increase in clinically significant CES-D and GEC scores compared to the AFE ≥12 group. This is the first study to find an association between the age children begin playing tackle football and later-life impairments. If replicated, these findings may have implications for safety recommendations for youth sports.

3

COCAINE ALTERS THE DAILY PATTERNS OF ADULT NEUROGENESIS

Stankiewicz, Alex; Akle, Veronica; Kopotiyenko, Konstantin; Yu, Lili; Fan, Sharon; Teng, Christina; Zhdanova, Irina

Anatomy and Neurobiology

Adult neurogenesis in vertebrates occurs in discrete regions of the central nervous system. The milieu specific to promoting proliferation in these neurogenic niches can vary in a circadian manner and might be fundamentally altered by pharmacologic agents, including drugs of abuse. To address this, we used the zebrafish model to determine whether adult neurogenesis undergoes circadian variation and if cocaine interferes with this process. The principal advantages of the model are abundant adult neurogenesis, strong circadian rhythms and established responsiveness to cocaine in this species. Moreover, zebrafish provides important benefits of a diurnal vertebrate model for translational circadian research into the effects of drugs of abuse in diurnal humans. Using BrdU labeling, expression levels for four cyclins and a kinase inhibitor that controls specific phases of the cell cycle, we documented a robust circadian rhythm of cell proliferation in adult brain. We then demonstrated two distinct effects of cocaine on this process. Acutely, cocaine augments the S phase of the cell cycle at the time of the circadian peak in neurogenesis, in the late evening. In contrast, the morning administration of cocaine lacks this acute effect but leads to reduced cell proliferation the night after. The results are consistent with and will be discussed in the context of our hypothesis of the “two-night circadian regulation of adult neurogenesis”.

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INHIBITORY INTERNEURONS IN THE ANTERIOR CINGULATE AND MEDIAL PREFRONTAL CORTEX IN PRENATALLY MALNOURISHED RATS

Wang X; Amaral AC; Mortazavi F; Rosene DL

Anatomy and Neurobiology

Prenatal protein malnutrition continues to be a significant problem in the world today. Exposure to prenatal protein malnutrition increases the risk of a number of neuropsychiatric disorders that are associated with inhibitory interneurons, including depression, schizophrenia and attention deficit hyperactivity disorder. Previous studies have found that neurons in anterior cingulate and medial prefrontal regions respond excessively to restraint stress in prenatally malnourished rats (Rosene et al., 2004). In this study, we investigate if prenatal protein malnutrition affects the subpopulation of inhibitory interneurons in the prefrontal cortex in relationship to the higher stress response. This was done using double-labeling immunohistochemistry with c-Fos to mark activated neurons and parvalbumin to mark inhibitory interneurons. Numbers of single and double-labeled neurons were quantified with unbiased stereology. Statistical analysis demonstrated that there was no effect of prenatal malnutrition on the total number of neurons or on the number of parvalbumin neurons. However, prenatal malnutrition was associated with a significant increase in the number of inhibitory parvalbumin positive neurons activated by restraint stress. This suggests that prenatal malnutrition altered the excitability of these inhibitory interneurons either directly or by altering their connectivity.

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COMPARATIVE STUDIES OF THE ENDOTHELIAL GLYCOCALYX LAYER IN THE AQUEOUS OUTFLOW PATHWAY OF HUMAN AND BOVINE EYES

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The glycocalyx on the vascular endothelium plays an important role in mechanotransduction and permeability. This study aims to compare the structure and distribution of glycocalyx in the bovine and human aqueous outflow pathways, where this layer remains uninvestigated. Enucleated bovine(N=6) and human(N=2) eyes were either immerse- or perfuse- fixed with 1% glutaraldehyde and 4% paraformaldehyde in DPBS and 0.05% Alcian Blue. Eyes were cut and immersed in 1% aqueous osmium tetroxide and 1% lanthanum nitrate followed by en-bloc staining then processed for electron microscopy. The glycocalyx distribution and thickness (in those regions where it was seen) were measured on the trabecular beams(TM), Schlemm's canal(SC)/aqueous plexus(AP), and collector channels(CC). The glycocalyx, which appears as a layer of hair-like brushes, was distributed non-uniformly in both bovine and human aqueous outflow pathways. However, the distribution was quite different in the two species. In bovine eyes, the fraction of the surface area covered with glycocalyx was found to be CC(37-75%) > TM(27-40%) > AP(5-28%), while in human eyes these fraction were found to be CC(59-81%) > SC(42-76%) > TM(15-34%). The glycocalyx was more uniform in the AP than TM in bovine eyes, whereas in human eyes, it was more uniform in SC than TM. Interestingly, in bovine eyes, the glycocalyx thickness was not significantly different at various regions (CC:80-113nm, AP:71-121nm, TM:62-127nm), whereas in human eyes, it was significantly thicker in CC(128-162nm) than SC(94-155nm), followed by TM(52-91nm). The implications of the glycocalyx in the aqueous outflow pathway remain to be clarified.

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ABSTRACT EXPRESSIONIST PAINTINGS REVEAL THE NEURAL SYSTEMS INVOLVED IN THE PROCESSING OF COLOR AND LUMINANCE: AN FMRI STUDY

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The field of visual neuroscience is concerned with how the brain perceives and processes visual information. In this study, we sought to identify regions of the brain involved in processing color and luminance information using functional magnetic resonance imaging (fMRI) and abstract paintings composed of color and luminance in different forms. The paintings used canonically and/or visually belong to the Abstract Expressionist genre, specifically Gesture Painting or Color Field Painting. Subjects were 15 healthy males and females with an average age of 27.5 years. They viewed 240 unique images in a block design. Half of the images were the original, saturated (i.e. color) forms of the paintings, and the other half were desaturated (i.e. grayscale) versions of the same paintings. In our analysis, we compared the saturated paintings to their desaturated counterparts – both as a group and within the Gesture and Color Field styles. We also compared the desaturated Gesture paintings to their saturated counterparts because many of the Gesture painters created work with little to no color. We found (1) the brain processes color in the Gesture and Color Field paintings differently (2) color in abstract form is capable of producing significant activation in the amygdala and (3) the color and luminance structures of the Gesture paintings produce different patterns of significant activation in the brain.

Program in Behavioral Neuroscience

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*

EPISODIC MEMORY AND EXECUTIVE FUNCTION IN FAMILIAL LONGEVITY

Andersen, Stacy

Behavioral Neuroscience

Successful aging, the ability to resist age-associated illnesses and functional disability, is of increasing importance as the population ages. Studies have shown that exceptionally long-lived individuals fit the successful aging paradigm by compressing disability toward the end of life. This study investigated whether there is evidence of successful cognitive aging in a familial longevity cohort, the Long Life Family Study (LLFS). A 2.5-hour neuropsychological battery emphasizing tests of episodic memory and executive function was administered to 70 proband generation and 100 offspring generation participants of the LLFS and 140 generation-matched referent participants without familial longevity. Comparison of LLFS proband generation participants with their referent cohort revealed no significant differences in test scores. LLFS offspring generation participants had borderline significant better performance on a test of executive function and attention compared with referents. These findings suggest that familial longevity is associated with better cognitive function even at relatively young ages. Modifiers of cognitive function including education, health habits, social networks, and cognitive reserve were assessed among participants in the proband generation. Education and participation in mid- and late-life cognitively stimulating activities were found to be higher in the referent cohort. This suggests that people without familial longevity may be more reliant on higher cognitive reserve in order to achieve similar cognitive performance to those from long-lived families. Implications of preserved cognitive function in long-lived families and the effect of cognitive reserve in those without familial longevity are discussed in terms of compression of disability and successful cognitive aging.

NEUROANATOMICAL AND COGNITIVE CORRELATES OF HIGH DRINKING LEVELS IN VETERANS DIAGNOSED WITH ALCOHOLISM AND PTSD

Maksimovskiy, Arkadiy L.; McGlinchey, Regina E; Fortier, Catherine B.; Salat, David H.; Milberg, William P.; Oscar-Berman, Marlene.Alcoholism frequently occurs in returning U.S. Veterans and is often comorbid with Post Traumatic Stress Disorder (PTSD). This study investigated the relationship between white matter abnormalities and neuropsychological performance in Operation Enduring Freedom and/or Operation Iraqi Freedom (OEF/OIF) alcoholic Veterans. Our two primary aims were: (1) to examine the relationship of alcoholism to brain structure and function, while controlling for the potential effects of comorbid PTSD, and (2) to examine whether the effects of drinking are moderated by the quantity of lifetime alcohol consumption. Our sample consisted of 71 deployed OEF/OIF Veterans stratified into four groups: alcoholics without PTSD, alcoholics with PTSD, participants with PTSD without comorbid alcoholism, and control participants without alcoholism or PTSD. Participants were given an exensive neuropsychological and psychiatric assessment battery, as well as Magnetic Resonance Diffusion Tensor Imaging (DT-MRI) scans. Results showed that disruption of executive functioning, and abnormal fractional anisotropy (FA; a measure of axonal integrity) within the frontal subcortical and dorsolateral frontal-parietal regions, occurred independently of the effects of PTSD. Furthermore, these cognitive and neuronal alterations were unique to the most severe subgroup of alcoholics who consumed the greatest amount of alcohol over the course of their lifetime, as compared to the rest of the sample. Axonal integrity within this subgroup, in regions underlying the frontal subcortical area, was shown to be decreased independently of cognitive changes. Integrity of axons underlying the dorsolateral frontal-parietal region, however, was increased. We hypothesized that the latter finding may be a compensatory mechanism for executive dysfunction.

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WHITE MATTER ALTERATIONS IN BLAST EXPOSURE AND BLAST-RELATED MILD TRAUMATIC BRAIN INJURY

Danielle R. Miller, Jasmeet P. Hayes, Ginette Lafleche, David H. Salat, Mieke Verfaellie

Behavioral Neuroscience

Due to the frequent use of improvised explosive devices in the Iraq and Afghanistan Wars, exposure to blast is common among OEF/OIF service members. In this study, we examined whether blast exposure with or without mTBI is associated with diffuse white matter changes and whether there is evidence for graded impairment.

Ninety-four OEF/OIF Veterans underwent DTI. Veterans were assigned to one of four groups based on clinical interview: no blast-exposure/no TBI, blast-exposure/no TBI, blast-exposure/mTBI without loss of consciousness (LOC), and blast-exposure/mTBI with LOC. Analyses focused on the number of clusters of white matter voxels with reduced fractional anisotropy relative to the no blast/no TBI group ("potholes"). Additionally, analyses considered the contribution of PTSD symptom severity as well as the association of neural findings with performance in five neuropsychological domains.

Analyses revealed that all blast-exposed groups, regardless of TBI, had a significantly greater number of potholes than the no blast/no TBI group. Furthermore, mTBI with LOC was associated with significantly more potholes than blast exposure alone. PTSD symptoms had no effect on the number of potholes. Finally, the number of potholes was a significant predictor of verbal memory performance, but not of performance in other cognitive domains.

These results suggest that blast exposure alone is associated with diffuse white matter integrity loss. Furthermore, individuals who experienced mTBI with LOC may be at particular risk for greater white matter loss. Additionally, the association between diffuse white matter abnormalities and verbal memory performance points to the clinical significance of these subtle neural changes.

Department of Biochemistry

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*

MECHANISMS OF AORTIC CARBOXYPEPTIDASE-LIKE PROTEIN REGULATION OF THE FIBROBLAST TO MYOFIBROBLAST TRANSITION

Tumelty, Kathleen E; Smith, Barbara D; Nugent, Matthew A; and Layne, Matthew D

Department of Biochemistry

Idiopathic pulmonary fibrosis (IPF) is a chronic, fatal, and incurable disease that causes the stiffening of lung tissue and gradual lung function decline. Fibrotic lungs are characterized by accumulation of smooth muscle α actin- (SMA) expressing myofibroblasts and excessive deposition of a collagen rich extracellular matrix. The differentiation of lung fibroblasts into myofibroblasts is stimulated by growth factors, including transforming growth factor β (TGFβ) and potentiated by a stiff mechanical environment. Our laboratory has identified a secreted matrix protein, aortic carboxypeptidase-like protein (ACLP), which is upregulated in IPF. ACLP knockout mice are protected from experimentally induced fibrosis. This led to the hypothesis that ACLP promotes the fibroblast to myofibroblast transition. ACLP expression preceded SMA and collagen I expression in differentiating primary mouse lung myofibroblasts. Recombinant ACLP induced SMA and collagen I expression in primary differentiating myofibroblasts and IMR90 human lung fibroblasts. ACLP knockdown by siRNA slowed myofibroblast differentiation and partially reverted differentiated myofibroblasts into fibroblasts. Because of the similarities among ACLP and TGFβ targets, it was hypothesized that ACLP stimulates TGFβ signaling. In lung fibroblasts, ACLP induced Smad3 phosphorylation and nuclear translocation, features of TGFβ signaling. The effects of ACLP on myofibroblast differentiation were dependent on TGFβ receptor (TβR) kinase activity and ACLP interacted directly with TβRII to promote myofibroblast differentiation. Additionally, ACLP modulated changes in differentiation between cells grown on softer versus stiffer matrices. These studies identified a novel mechanism of ACLP action in fibroblasts and may lead to new therapeutic strategies to treat fibrotic disease.

THE ROLE OF CYTOSKELETON SIGNALING IN CONTROLLING THE COMMITMENT OF PROGENITORS TO ADIPOSE LINEAGE VERSUS OSTEOBLAST LINEAGE

Hejiao Bian, Matthew Layne, Barbara Smith, Stephen Farmer

Arising from common progenitors in the bone marrow, adipogenesis and osteogenesis are closely associated yet mutually exclusive during mesenchymal stem cell (MSC) development. Previous studies have shown that the morphological changes can affect the early commitment of pluripotent mesenchymal stem cells to adipose lineage versus osteoblast lineage via modulation of RhoA activity. The RhoA pathway regulates actin polymerization dynamics to promote the incorporation of Globular-actin (G-actin) into Filament-actin (F-actin). In doing so, Myocardin-related transcription factors (MRTFs) bound with G-actin are released for nuclear import to co-activate Serum Response Factor (SRF) cytoskeletal target genes. Exactly how the RhoA-actin-MRTF-SRF circuit is involved in the regulation of early commitment of mesenchymal stem cells was investigated in this study.

Preliminary results showed that MRTFA and SRF inhibits adipogenesis and enhances osteogenesis in MSCs, whereas dominant-negative MRTFA and SRF had the opposite effects. Bone marrow stem cells isolated from global MRTFA knockout mice showed increased adipogenesis and compromised osteogenesis when compared to WT littermates. The SRF inhibitor, CCG1423, mimicked the effects of knocking out MRTFA in WT mouse bone marrow stem cells by inhibiting osteogenesis and promoting adipogenesis. MRTFA global KO mice also showed smaller whole body weight, shorter femoral and tibial lengths as well as significantly decreased trabecular bone volume in their femur with decreased osteogenic genes levels and the IGF-1level. MRTFA and SRF appear to be crucial regulators of the balance between adipogenic and osteogenic differentiation of the bone marrow stem cells.

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TAZ/YAP DIRECT TGFβ-INDUCED TUMORIGENIC PHENOTYPES IN BREAST CANCER CELLS

Hiemer, Samantha E.; Szymaniak, Aleks; Varelas, Xaralabos

Department of Biochemistry

Uncontrolled Transforming growth factor-beta (TGFβ) signaling promotes aggressive metastatic properties in late-stage breast cancers. However, how TGFβ-mediated cues are directed to induce late-stage tumorigenic events is poorly understood, particularly given that TGFβ has clear tumor suppressing activity in other contexts. Here we demonstrate that the transcriptional regulators TAZ and YAP (TAZ/YAP), key effectors of the Hippo pathway, are necessary to promote and maintain TGFβ-induced tumorigenic phenotypes in breast cancer cells. Interactions between TAZ/YAP, TGFβ-activated SMAD2/3, and TEAD transcription factors reveal convergent roles for these factors in the nucleus. Genome-wide expression analyses indicate that TAZ/YAP, TEADs and TGFβ-induced signals coordinate a specific pro-tumorigenic transcriptional program. Importantly, genes cooperatively regulated by TAZ/YAP, TEAD, and TGFβ, such as the novel targets NEGR1 and UCA1, are necessary for maintaining tumorigenic activity in metastatic breast cancer cells. Nuclear TAZ/YAP also cooperate with TGFβ signaling to promote phenotypic and transcriptional changes in non-tumorigenic cells to overcome TGFβ repressive effects. Our work thus identifies crosstalk between nuclear TAZ/YAP and TGFβ signaling in breast cancer cells, revealing novel insight into late-stage disease-driving mechanisms.

DISCOVERY OF A NOVEL PEELING REACTION THAT CONTRIBUTES TO THE UNDERESTIMATION OF 3-*O*-SULFATION IN HEPARAN SULFATE

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The heparan sulfate (HS) 3-*O*-sulfation is an important modification that determines the HS/heparin binding specificity of antithrombin III and plays a key role in herpes simplex virus (HSV) infection. However, HS 3-*O*-sulfation is a rare modification that has an extremely low natural abundance and therefore there have been very few functional studies other than the two cases above. This is in sharp contrast to the fact that up to seven distinct isoforms of 3-*O*-sulfotranserases have been discovered in mammals. Here we describe mass spectrometric determination of a novel peeling reaction that specifically degrades HS chains with 3-*O*-sulfated glucosamine at the reducing-end, which contributes to the underestimation of this important sulfation.

In the process of purifying oligosaccharides from lyase digests of porcine intestinal mucosa HS, we noticed altered abundances of chromatographic peaks that occur over time. The extent of the changes increased as pH was raised over 7.0. The corresponding peaks were confirmed by tandem mass spectrometry showing the loss of a 3-*O*-sulfated glucosamine residue at the reducing end, a reaction similar to peeling. We showed this reaction occurs in synthetic 3-*O*-sulfated heparin saccharide and enzymatically modified synthetic oligosaccharide with 3-*O*-sulfation and compared susceptibility of the 3-*O*-sulfation modified product with the substrate. To assess the extent to which peeling occurs using typical methods for heparin/HS depolymerization and workup, we studied a commercial low molecular weight heparin drug, Enoxaparin and confirmed the peeling on the tetrasaccharides containing 3-*O*-sulfation, which are routinely used to gauge the anticoagulant activity of their preparations.

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MYOCARDIN-RELATED TRANSCRIPTION FACTORS REGULATE THE DEVELOPMENT OF BEIGE ADIPOCYTES IN WHITE ADIPOSE TISSUE

Chendi Li, Meghan E. McDonald, Hejiao Bian, Matthew D. Layne, Barbara D. Smith and Stephen R. Farmer

Enhancement of beige adipocytes in white adipose tissue (WAT) is a potential therapeutic approach to combat obesity and type 2-diabetes. This can be achieved by stimulating beige cell development from progenitors. Disruption of actin cytoskeleton was shown to facilitate commitment of mesenchymal stem cells (MSCs) to the adipogenic lineage. Polymerization of actin promotes the transcriptional activity of myocardin-related transcription factor (MRTF)-serum response factor (SRF) nuclear complexes. SRF was shown to inhibit adipogenesis, so we hypothesized that inhibition of MRTF-SRF signaling promote beige adipocyte development. Our results showed that BMP7, a known inducer of brown adipogenesis, disrupted the actin cytoskeleton in 10T1/2 MSCs leading to attenuation of MRTF-SRF activity. Treatment of 10T1/2 MSCs with pharmacological inhibitor of MRTF-SRF signaling prior to their differentiation into adipocytes selectively induced brown adipose (BAT) genes with no change in expression of classic adipogenic genes. These *in vitro* data suggested that inhibition of actin-MRTF-SRF signaling in MSCs might selectively promote beige adipocyte formation. Strikingly, MRTFA knock out (KO) mice have significantly more beige cells within the inguinal (ING) WAT compared to wild type (WT) mice. Stromal cells derived from the ING WAT of KO mice undergo *in vitro* brown adipogenesis more efficiently than WT cells indicating that MRTFA is functioning in an autonomous manner. MRTFA KO mice are resistant to Diet Induced Obesity compare to their WT littermate. These findings reveal a novel role for the MRTFA-SRF pathway in beige adipocyte development and they have the potential of leading to the development of novel anti-obesity therapeutics.

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THE P2X7 RECEPTOR IN CORNEAL EPITHELIAL WOUND HEALING

Minns, Martin S., Rich, Celeste B., and Vickery Trinkaus-Randall, Ph.D.
Biochemistry

Proper wound healing is essential in corneal epithelium, as delayed healing can lead to infection and loss in vision quality. We have identified the P2X7 purinergic receptor as an important promotor of corneal epithelial healing. However, the mechanism by which P2X7 effects wound healing is still unknown. Our goal is to determine the role of P2X7 in corneal wound repair. To test this, we examined both human corneal epithelial cells and intact rat corneas in organ culture. We performed real-time PCR, western blot analysis and immunofluorescent analysis using confocal microscopy at specific times after injury.

We made 3mm-diameter debridement wounds in rat corneas and placed them in culture to heal. Epithelial wounds completely closed within 24 hours after injury. P2X7 total mRNA and protein levels decreased shortly (< 1 hour) after injury and remained low during healing. Immunofluorescent analysis of the wounds indicated that P2X7 protein expression was increased in the first few cells at the leading edge but decreased in cells further away from the edge. Under high glycemic conditions P2X7 mRNA was elevated in the unwounded epithelium and wound healing was significantly delayed. When control cultures were treated with oxidized ATP there was also a reduction in wound repair rate. These data show that P2X7 is necessary for proper healing, but excessive levels can also inhibit healing.

THE ROLE OF CAVEOLIN1 IN P2X7 REGULATION DURING CORNEAL WOUND HEALING

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In the cornea, physical injury causes the release of nucleotides, which bind to purinergic receptors and induce downstream signaling events leading to directional cell migration[1]. The exact mechanisms behind caveolin1 (cav1) involvement in migration and wound healing have yet to be determined. Data suggests that Rac1 recruits cav1 to focal adhesion sites allowing cav1 to tag Rac1 for degradation[2]. Phosphorylated cav1 activates Csk which inhibits Src kinase, causing delayed wound healing, Rac1 inhibition, and Rho activation[3]. Further data shows that in cav1 KO mice, $P2X_7$ expression was strongly decreased[4].

Our preliminary data shows that $P2X_7$ inhibition impairs wound healing in human corneal limbal epithelial cells (HCLEs). When HCLEs are stimulated by BzATP both $P2X_7$ and cav1 expression strongly increase. Our confocal data indicates that $P2X_7$ and cav1 are present in the same region of the cell, but do not appear to co-localize, as seen in other cell types. Our preliminary hypothesis is that cav1 regulates $P2X_7$ expression possibly through interaction with a linker protein, thereby allowing cav1 to control corneal wound healing.

References:

1. Weinger I. *et al.* Tri-nucleotide receptors play a critical role in epithelial cell wound repair. *Purinergic Signalling* (2005) 1: 281-292.

2. Nethe M. *et al.* Focal-adhesion targeting links caveolin-1 to a Rac1-degradation pathway. *Journal of Cell Science* (2010) 123: 1948-1958.

3. Grande-Garcia A. *et al.* Caveolin-1 regulates cell polarization and directional migration through Src kinase and Rho GTPases. *Journal of Cell Biology* (2007) 177(4): 683-694.

4. Bart K. *et al.* Caveolin-1 influences $P2X_7$ receptor expression and localization in mouse lung alveolar epithelial cells. *FEBS Journal* (2007) 274: 3021-3033.

NEW MECHANISMS FOR VEGF-A REGULATION BY HEPARAN SULFATE

Teran, Madelane; Nugent, Matthew A

Biochemistry

Angiogenesis is a highly regulated process orchestrated by the vascular endothelial growth factor (VEGF) system, comprised of multiple isoforms and cell surface receptors. In addition, heparin/heparan sulfate (HS) proteoglycans and neuropilins (NRP) have been identified as co-receptors, yet the mechanism of action has not been defined. In the present study we characterized molecular interactions between the most commonly expressed VEGF isoforms and their receptors and co-receptors, using surface plasmon resonance (SPR) as well as other in vitro binding assays. Our data suggest a new mechanism for heparin/HS to regulate VEGF function independent of direct binding to $VEGF_{165}$ or VEGFR2. We have shown that VEGFR1 and NRP1 bind heparin independently of $VEGF_{165}$ and that these interactions can selectively control VEGF-receptor complex formation. We propose that HS/heparin and NRP1 dictate the specific receptor type activated by VEGF and ultimately determine the biological response of the VEGF system. The ability of these co-receptors to fine-tune VEGF responsiveness suggest the possibility that VEGF-mediated angiogenesis can be selectively stimulated or inhibited by targeting HS/heparin and NRP1. Supported by HL088572 and AHAF M2012014.

Estimated Expenses

Conference application and lodging $750

Travel $100

Total $100

Program in Genetics and Genomics

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*

LOSS OF *ZEB2* CAUSES GLOMERULOCYSTIC KIDNEY DISEASE IN MICE

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BACKGROUND: ZEB2 is a Zinc finger E-box-binding homeobox transcription factor. Mutations in *ZEB2* cause the Mowat-Wilson syndrome, an autosomal dominant disorder characterized by multiple congenital anomalies including kidney anomalies. However, the role of the *ZEB2* gene in kidney development is unknown.

METHODS: We generated kidney specific *Zeb2* conditional knockout mice by crossing *Zeb2* floxed allele (*Zeb2*$^{flox/flox}$) with the *Pax2*-cre and *Six2*-cre alleles. The conditional knockout mice were analyzed from embryonic stage E14.5 to postnatal 8 weeks old. The mice kidneys were analyzed by H&E staining and immunostaining for apoptosis and cell proliferation. Gene expression analysis was performed by TaqMan assays. Kidney function was assessed by measuring urine protein levels, serum creatinine and blood urea nitrogen.

RESULTS: Both *Zeb2* conditional knockout mice developed kidney glomerular cysts after E16.5 days. *Zeb2*$^{flox/flox}$;*Pax2*-cre mice died at birth and *Zeb2*$^{flox/flox}$;*Six2*-cre mice developed abnormal kidney function at 5 weeks old. The cysts originate from the glomeruli with dilated Bowman's capsule and collapse of glomerular tufts. Reduced apoptosis and increased proliferation were detected in *Zeb2* knockout mice compared to wild type controls. Gene expression analysis revealed increased level of *Pkd1* mRNA in the developing kidney of both *Zeb2* conditional knockout mice compared to the wild-type controls. Interestingly, an E-box binding domain has been identified in the promoter region of *Pkd1* gene and overexpression of *Pkd1* has been reported to cause glomerular cysts in mice.

CONCLUSION: *Zeb2* regulates gene expression of *Pkd1* in the developing kidney and loss of *Zeb2* causes glomerulocystic kidney disease in mice.

GENE AND MIRNA EXPRESSION NETWORKS SPECIFIC TO NEVER SMOKER LUNG ADENOCARCINOMA

Garrison, Carly; Kusko, Rebecca; Wang, Teresa; Campbell, Josh; Perez-Rogers, Joseph; Luo, Lingqi; Beane, Jennifer; Liu, Gang; Kadara, Humam; Belinsky, Steven; Lenburg, Marc E.; Spira, Avrum.

GPGG

While smoking is a major risk factor for lung cancer, there is a growing incidence of lung cancer in never smokers. Never smokers (NS) who develop lung cancer exhibit disparate profiles of somatic mutations and clinical responses to targeted therapy relative to lung cancer arising in current or former “ever” smokers (ES). We sought to characterize transcriptomic differences specific to NS adenocarcinoma (AdC) to gain insights into the molecular differences underlying NS and ES AdC carcinogenesis.

Total RNA was isolated from matched pairs of lung AdC tumor and adjacent normal tissue from 22 subjects (8NS, 14ES). RNA libraries were sequenced on the Illumina HiSeq 2000. Tumor-specific expression differences between NS and ES were identified using linear mixed-effects ANOVA. MiRConnx was used to construct miRNA-mRNA networks.

We identified 120 mRNA and 15 miRNA whose expression was modified uniquely in NS AdC. Several of the differentially expressed mRNA and miRNA were validated by qRT-PCR in samples from an independent cohort.

In summary, the construction of a miRNA-mRNA regulatory network has enabled us to identify molecular alterations that may be specific to NS lung AdC. Ultimately, these findings may serve to broaden the landscape of personalized therapeutic and treatment options by identifying targetable molecular interactions and therapeutic drug candidates for lung AdC in never smokers.

MicroRNAs Located in the Hox Gene Clusters Are Implicated in Huntington's Disease Pathogenesis

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Transcriptional dysregulation has long been recognized as central to the pathogenesis of Huntington's disease (HD). MicroRNAs (miRNAs) represent a major system of post-transcriptional regulation, by either preventing translational initiation or by targeting transcripts for storage or for degradation. Using next-generation miRNA sequencing in prefrontal cortex (Brodmann Area 9) of twelve HD and nine controls, we identified five miRNAs (miR-10b-5p, miR-196a-5p, miR-196b-5p, miR-615-3p and miR-1247-5p) up-regulated in HD at genome-wide significance (FDR q-value <0.05). Three of these, miR-196a-5p, miR-196b-5p and miR-615-3p, were expressed at near zero levels in control brains. Expression was verified for all five miRNAs using reverse transcription quantitative PCR and all but miR-1247-5p were replicated in an independent sample (8HD/8C). Ectopic miR-10b-5p expression in PC12 HTT-Q73 cells increased survival by MTT assay and cell viability staining suggesting increased expression may be a protective response. All of the miRNAs but miR-1247-5p are located in intergenic regions of Hox clusters. Total mRNA sequencing in the same samples identified fifteen of 55 genes within the Hox cluster gene regions as differentially expressed in HD, and the Hox genes immediately adjacent to the four Hox cluster miRNAs as up-regulated. Pathway analysis of mRNA targets of these miRNAs implicated functions for neuronal differentiation, neurite outgrowth, cell death and survival. In regression models among the HD brains, huntingtin CAG repeat size, onset age and age at death were independently found to be inversely related to miR-10b-5p levels. CAG repeat size and onset age were independently inversely related to miR-196a-5p, onset age was inversely related to miR-196b-5p and age at death was inversely related to miR-615-3p expression. These results suggest these Hox-related miRNAs may be involved in neuroprotective response in HD. Recently, miRNAs have shown promise as biomarkers for human diseases and given their relationship to disease expression, these miRNAs are biomarker candidates in HD.

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THE ROLE OF CELL POLARITY IN THE ENGULFMENT OF DYING GERM CELLS.

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Graduate Program in Genetics and Genomics

As organisms develop, multiple processes need to occur for tissue specification and organization. One is the establishment of cell polarity, which drives cell fate specification. Another is programmed cell death, to remodel tissue and clear damaged or diseased cells from the body. During Drosophila oogenesis, programmed cell death can be induced to prevent egg chambers from maturing under low nutrient conditions. When this occurs, the germline dies and is cleared by a process known as engulfment. Somatic follicle cells (FCs) surrounding the germline synchronously enlarge and engulf the corpses of the dying germline cells. It is unknown what triggers the enlargement of the FCs or if this enlargement has an energy requirement. Previous research has shown that the apical side of a FC is heavily marked by cell polarity proteins to specify the apical side. We hypothesize that cell polarity plays a role in the enlargement of the FCs during engulfment. To test this, we first examined whether FC polarity is maintained in mutant fly lines known to have engulfment defects. We then examined RNAi lines for genes responsible for the maintenance of FC polarity to determine if there were defects in engulfment. To determine if there is an energy requirement we examined an RNAi line of complex V of the electron transport chain. We next assessed FC enlargement directly, using RNAi lines that target the hippo signaling pathway. These studies could uncover a new role for cell polarity in engulfment and new pathways required for FC enlargement.

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COMPREHENSIVE GENOMIC PROFILING OF THE LUNG TRANSCRIPTOME IN EMPHYSEMA AND IPF USING RNA-SEQ

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As part of the Lung Genomics Research Consortium, we characterized transcriptomic changes underlying the molecular pathogenesis of Emphysema (Emp) and Idiopathic Pulmonary Fibrosis (IPF) using RNA-Seq, and integrated these changes with mRNA and microRNA microarray expression data from the same samples. Sequencing was performed on 87 lung tissue samples using 75nt paired end reads on the Illumina GAIIx. Using a subset of subjects with IPF (n=19), Emp (n=19), or control (n=20) differential expression was determined. The expression levels of 1770 genes differed between IPF and Control, and 220 genes between Emp and Control ($p<0.001$). Genes that go up in both Emp and IPF are enriched for the p53/hypoxia pathway. These results were validated by IHC of select p53/hypoxia genes and GSEA analysis of microarrays of independent samples. Using MirConnX we identified several miRNA that anticorrelated with genes differentially expressed in both IPF and Emphysema. miRNA overexpression in cell lines followed by PCR was used to validate several connections. Gene expression arrays run on overexpression of mir-96 revealed enrichment of upregulated IPF genes. Using reads aligned across known splice junctions, we identified 5 Emp associated isoforms and 19 IPF-associated isoforms ($p<0.01$). These findings may ultimately lead to novel biomarkers for chronic lung disease and identification of potential novel therapeutic targets.

MAPPING THE AIRWAY-WIDE MOLECULAR FIELD OF INJURY IN SMOKERS WITH LUNG CANCER

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Genetics and Genomics

Identifying effective early detection biomarkers is crucial for improving lung cancer clinical management. We have additionally demonstrated that gene-expression profiles in cytologically normal mainstem bronchial epithelium can serve as an early diagnostic biomarker for lung cancer. Here we expand on our previous work by spatially mapping the molecular field of injury throughout the entire respiratory tract in smokers with lung cancer. Using Affymetrix Gene ST 2.0 arrays, we profiled genome-wide gene-expression in 1) lung lesions and adjacent normal lung obtained from smokers undergoing surgical resection, 2) epithelial brushings obtained at intraoperative bronchoscopy from the nasal epithelium, main carina and ipsilateral and contralateral proximal and distal bronchi (relative to the location of the resected lung lesion), and 3) epithelial brushings obtained at lobectomy from sub-segmental bronchus (adjacent to tumor). Linear modeling approaches comparing the airways and tumors of patients with cancer to those with benign lung disease were used to explore relationships in cancer-specific gene-expression alterations across sites within the respiratory tract. Furthermore, a linear mixed effects model uncovered genes and pathways which change in expression in a gradient-like manner as distance from the tumor increases. Our findings suggest that the molecular field of injury encompasses airway-wide alterations throughout the entire respiratory tract of smokers with lung cancer as well as gradient profiles that change with respect to proximity of the nearby tumor.

EPIGENETIC REGULATION OF GROWTH HORMONE-RESPONSIVE, SEX-BIASED GENES IN ADULT MOUSE LIVER

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Sex-dependent pituitary growth hormone (GH) secretory patterns determine the sex-biased expression of ~1,000 genes in mouse liver, affecting drug metabolism and liver disease. In males, GH is secreted in a highly pulsatile manner with virtually no plasma GH detectable between pulses. In females, GH secretion is more frequent, resulting in the near continuous presence of GH in circulation. Continuous GH infusion of intact male mice for 14 days overrides the endogenous male, pulsatile plasma GH pattern and down-regulated 55% (208/380) of male-biased genes while up-regulating 51% (213/419) of female-biased genes, as shown by RNA-seq. Genes that respond early (10hr GH treatment) include *Cux2*, a female-specific transcription factor that represses male-biased genes and induces female-biased genes. Chromatin immunoprecipitation (ChIP) demonstrated that histone-H3 lysine-27 acetylation (H3K27ac), a chromatin mark associated with active enhancers, shows a time-dependent responsiveness to GH treatment that coincides with or precedes the associated changes in expression of sex-biased genes. Histone-H3 lysine-27 trimethylation (H3K27me3), a sex-biased repressive mark found at highly female-biased genes, showed decreases that coincide temporally with the de-repression of female-biased gene expression in response to GH treatment. Open chromatin regions, which encompass a large fraction of regulatory elements genome-wide, were identified using global DNase I hypersensitivity analysis. Overall, the chromatin accessibility at male-biased hypersensitivity sites decreased while the accessibility of female-biased sites increased, suggesting a role for these sites in sex-differential, GH-regulated gene expression. Further integration of these GH-differential histone mark and chromatin accessibility maps with gene expression data will help elucidate global transcriptional and epigenetic networks that dictate sex-biased liver gene expression.

DISSECTING THE SMAD4 METASTASIS SUPPRESSOR COMPLEX TO IDENTIFY NOVEL THERAPEUTIC TARGETS & PROGNOSTIC MARKERS IN COLON CANCER

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Despite early detection through routine screening with colonoscopy, colon cancer remains the second leading cause of cancer mortality in men and women combined in the United States, mostly attributed to metastasis. Hence, it is essential to elucidate the molecular mechanisms underlying metastasis, identify druggable targets, and develop prognostic markers that can predict disease progression and stratify patients for therapy based on these markers. In advanced colon cancer, there is frequent loss of heterozygosity (LOH) at chromosome 18q21; interestingly, *SMAD4*, a tumor suppressor that plays a central role in the canonical TGF-beta anti-growth signaling pathway, is localized to this region. Previously, we have shown that SMAD4 can inhibit hypoxia-inducible factor 1 alpha (HIF1-alpha) to suppress various malignant phenotypes, whereas inactivation of SMAD4 in colon cancer enhances cell migration, increases the expression of VEGF, GLUT1, and MMP9, and promotes resistance to 5'-fluorouracil. Based on this study, we hypothesized that SMAD4 interacts with transcription factors and cofactors to form a complex that negatively regulates metastasis. We have generated Flag- and HA-tagged SMAD4 proteins and plan to identify co-immunoprecipitated proteins using mass spectrometry. The functional roles of these proteins in regulating metastasis will be characterized in experimental models of cancer progression and their contribution to human colon cancer assessed through *in silico* analysis of gene expression profiles and examination of clinical specimens at different stages of disease progression. We suggest that the genes corresponding to the proteins in this complex may be potentially useful in predicting disease progression, and the gene products may eventually serve as therapeutic targets to treat metastatic colon cancer.

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*

THE LUNG-LIVER AXIS FACILITATES INNATE IMMUNITY AND SURVIVAL DURING PNEUMONIA

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During pneumonia, a systemic acute phase response (APR) occurs concomitantly with local innate immunity in the lungs. However, it is unknown whether or how circulating liver-derived acute phase proteins (APPs) fortify pulmonary host defense. To address this, we intratracheally infected APR-null mice with mild or severe doses of *E. coli*. APR-null mice lack hepatocyte STAT3 and RelA, which ablates virtually all transcriptional changes during pneumonia. Serum APPs were unaffected by pneumonia in mutants, despite significant changes in WT controls. Severely infected APR-null mice exhibited increased mortality and liver injury. Liver injury, but not survival, was improved by neutralizing TNFα. Lung injury was extreme and equivalent between genotypes, but bacterial clearance was compromised in APR-null mutants. Following a milder, non-lethal *E. coli* dose, APPs were uniformly decreased in mutant bronchoalveolar lavage fluid, in association with decreased lung inflammation and cytokine expression. Airspace macrophages produced fewer cytokines in mutant mice, suggesting a pivotal role for macrophage activation by liver-derived APPs. We propose that the hepatic APR influences both extra- and intra-pulmonary responses to lung infection. While hepatic signals directly curb survival and liver injury induced by TNFα, liver-derived APPs downstream of these same signals promote inflammation and host defense in the lungs.

MECHANISTIC DIFFERENCES IN HIV-1 AND HIV-2 INTERACTIONS WITH DENDRITIC CELLS

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Microbiology

HIV-2 infection has been restricted predominantly to West Africa; in contrast HIV-1 has spread rapidly and accounts for 95% of all HIV infections globally. Interestingly, HIV-2 infected individuals maintain low viral loads and have lower mortality rates than HIV-1 infected individuals. The mechanistic basis for viral control and slower progression to AIDS in HIV-2 infected individuals remains unclear. We hypothesized that differential interaction of HIV-2 with myeloid dendritic cells (DCs) plays an important role in its restricted pathogenesis. DCs are sentinel cells that not only express pathogen recognition receptors that sense virus and activate innate immune responses but also restriction factors such as SAMHD1 that attenuate HIV-1 infection by inhibiting reverse transcription. We have previously shown that HIV-1 evades cell-intrinsic restrictions in DCs by exploiting the receptor siglec 1 (CD169) for enhanced transfer to CD4+ T lymphocytes without establishing productive infection in DCs. In contrast, HIV-2 encodes an accessory protein, Vpx that can target SAMHD1 for degradation resulting in robust infection of DCs. Interestingly, production of IP-10 (interferon inducible protein-10), a biomarker for activation of type I IFN signaling pathways, was elevated in mRNA and protein in HIV-2, but not HIV-1, exposed DCs. We show that even though HIV-2 establishes infection in DCs, the virus derived from DCs is attenuated in its spread to CD4+ T cells. We conclude that productive HIV-2 infection of DCs can result in detection of viral replication intermediates by DC-specific sensors that trigger antiviral signaling cascades, attenuate virus infectivity and hence inhibit DC-mediated HIV-2 dissemination.

POST-TRANSCRIPTIONAL REGULATION OF RNA DURING EBOLA VIRUS INFECTION

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Ebola virus (EBOV) is a nonsegmented negative sense (NNS) RNA virus that causes severe hemorrhagic fever in humans with case fatality rates up to 90%. While immune responses to EBOV are often studied, less is known about the impact of post-transcriptional RNA regulation on EBOV replication and pathogenesis. The stability of many cellular mRNAs (particularly cytokines) is often regulated by AU-rich elements (AREs) located within their 3'untranslated regions (3'UTR). These cis-acting elements are regarded as stability elements that are recognized by RNA-binding proteins that influence mRNA degradation. Two prototypic ARE-binding proteins are HuR and TTP. Because the EBOV RNA genome is highly AU-rich (about 70%) and four of the seven EBOV mRNAs contain putative AREs within their 3'UTRs, we examined if this type of RNA regulation plays a role during EBOV infection. Using a 3'UTR luciferase reporter assay, we found that TTP negatively targeted the 3'UTR of the EBOV nucleoprotein (NP). When a full length NP expression plasmid was co-transfected into cells with TTP, there was a significant decrease in both NP protein expression and mRNA quantity. Using the EBOV minigenome system, we saw that TTP had negative effects on EBOV replication/transcription. We next examined the location of HuR and TTP during infection, and saw that HuR was sequestered within viral inclusions, while TTP was excluded. We therefore hypothesize that the virus selectively sequesters cellular RNA binding proteins such as HuR, to benefit viral replication, and excludes other proteins such as TTP, which may have negative effects on viral replication/ transcription.

EXAMINATION OF ANTIBODY DYNAMICS AFTER VACCINATION AGAINST ANTHRAX

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Department of Microbiology

The introduction of vaccination was a paradigm shifting event in modern healthcare. A major mechanism of protection afforded by vaccination is the development of pathogen-specific antibodies. During the course of repeated vaccination with the same immunogen, as occurs in the delivery of a vaccine series, affinity maturation leads to the development of antibodies with increased affinity for the immunizing agent. However, the mutation and selection events during this process are not well understood. We have used recent advances in next-generation sequencing to investigate individual antibody repertoires throughout vaccination. We have followed adult volunteers receiving 5 doses of Anthrax Vaccine Adsorbed (AVA) and analyzed B cell immunoglobulin heavy chains. Our lab has developed sophisticated statistical tools to analyze the diverse V-D-J region that encodes for the variable region of antibodies. From our data, we can infer affinity maturation events within clonally related families, which may have future predictive value for functionally important mutations. In addition, our data also indicate that clonal lineages may be more dynamic than previously recognized, with naïve, memory and plasmacyte B cells all regularly preceding expansion events. Further investigation into these observations will potentially contribute to the future development of improved vaccines.

THE PIRNA BINDING PROTEIN MIWI2 IS INDUCED DURING INFECTION TO ENHANCE CYTOKINE EXPRESSION

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PIWI proteins associate with PIWI-interacting RNAs (piRNAs) in the mammalian germ line, and function to repress ancient retroviral elements. Ablation of any of the three PIWI proteins in mice (MIWI, MILI, or MIWI2) results in defects in spermatogenesis due to aberrant expression of LINE elements. While the germ cell functions of PIWI proteins have been described, next to nothing is known about their potential role(s) in somatic cells. To our surprise, microarray analysis and qRT-PCR of sorted alveolar epithelial cells demonstrated that a single PIWI protein, MIWI2 is induced in the lung during bacterial pneumonia. To determine the genes regulated by MIWI2, we treated an epithelial cell line with MIWI2 or control targeting shRNA, stimulated with TNFα and profiled gene expression by microarray. Nine genes were significantly changed in MIWI2 depleted compared to control cells (FDR<0.1). Among the genes decreased are cytokine genes including IL-6, GITRL, and CXCL7, all known to be important during the immune response to bacterial pneumonia. To determine whether MIWI2 could impact innate immune gene expression *in vivo,* we infected MIWI2 knockout mice with *Streptococcus pneumoniae* intratracheally and analyzed cytokine expression in bronchoalveolar lavage fluid. Excitingly, MIWI2-deficient animals exhibited a reduction in airspace IL-6 concentration after pneumonia. Collectively the results of these integrated studies indicate that MIWI2 is capable of enhancing cytokine expression. Given the known role of MIWI2 as a piRNA binding protein, future studies will determine whether somatic piRNAs or other non-coding RNAs are retasked to regulate pro-inflammatory cytokine expression. To our knowledge this is the first report of MIWI2 being induced to regulate inflammatory gene mediators in somatic cells.

Program in Molecular Medicine

Participants

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*

AN ADENOSINE RECEPTOR-KRUPPEL-LIKE FACTOR 4 AXIS INHIBITS ADIPOGENESIS

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Adipogenesis represents a key process in adipose tissue development, and remodeling including during obesity. Exploring the regulation of adipogenesis by extracellular ligands is fundamental to our understanding of this process. Adenosine, an extracellular nucleotide signaling molecule, found in adipose tissue depots acts on adenosine receptors. Here, we report that among these receptors, the A2b adenosine receptor (A2bAR) is highly expressed in adipocyte progenitors. Activation of the A2bAR potently inhibits differentiation of mouse stromal-vascular cells into adipocytes, while A2bAR knockdown stimulates adipogenesis. The A2bAR inhibits differentiation through a novel signaling cascade involving sustained expression of Kruppel-like factor 4 (KLF4), a regulator of stem cell maintenance. Knockout or knockdown of KLF4 ablates the ability of A2bAR to inhibit differentiation. A2bAR activation also inhibits adipogenesis in a human primary preadipocyte culture system. We analyzed the A2bAR-KLF4 axis in adipose tissue of obese subjects, and intriguingly found a strong association between A2bAR and KLF4 expression in both subcutaneous and visceral human fat. Hence, our study implicates the A2bAR as a regulator of adipocyte differentiation and the A2bAR-KLF4 axis as a potentially significant modulator of human adipose biology.

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A FUNCTION OF THE NEUROENDOCRINE SYSTEM IN MUCOUS HYPERPLASIA

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Childhood asthma is steadily increasing worldwide. Risk factors include early-life airway insults by pollutants, cigarette smoke, respiratory viral infection, and allergen exposure. Clinical hallmarks of asthma include T_H2 inflammation, airway hyper-reactivity and mucous hyperplasia. Mucous hyperplasia is a major contributor to cause of death in patients suffering from asthma. Available drugs only alleviate airway inflammation and bronchoconstriction but to develop better therapies for asthma, the mechanism behind mucous overproduction needs to be fully understood.

Mucus serves as a first line of defense to protect and clear the lung from potential pathogens or toxic substances. In asthma, however, mucous hyperplasia results in airway obstruction contributing to mortality. In addition to inflammatory signals, mucous hyperplasia in mouse models of asthma requires GABA, a central neurotransmitter. Our preliminary studies show that neuroendocrine bodies (NEBs) are the only source of GABA in the airway. In addition, my lab found that the neurotrophin 4 (NT4)-dependent innervation of NEBs is required for GABA signaling in early-life allergen-induced mucous hyperplasia. Built upon a paradigm that the neuroendocrine system mediates the communication between the nervous system and endocrine secretion to control important body functions, these preliminary findings implicate an unrecognized role of NEBs in mediating neural control of endocrine GABA secretion in the airway. **I hypothesize that the GABAα and GABAβ pathways have distinct roles in lung epithelial trans-differentiation and Muc5ac gene expression after early-life allergen exposure.** Results of my proposed study may identify new drug targets for the treatment of mucous hyperplasia in asthma.

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CONTROL OF VEGF-MATRIX-ENDOTHELIAL RESPONSE BY ECM STIFNESS

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Vascular endothelial growth factor (VEGF) drives endothelial cell maintenance and normal/ pathological angiogenesis. Cells contact surface mechanics have profound effects on endothelial cell behavior and signaling, suggesting that VEGF activity might be influenced by cell-substrate mechanics. We studied VEGF binding, internalization, and signaling as a function of substrate stiffness using endothelial cells cultured on Fibronectin linked polyacrylamide gels. To further investigate the role of VEGF binding to the endothelial ECM, we pre-treated the Fibronectin ECM with heparin, exposing a cryptic VEGF binding region. The softest substrates were least able to respond to heparin, yet surprisingly, they were the most sensitive to VEGF after heparin treatment resulting in increased receptor binding and internalization. These alterations were mediated by VEGFR2 and were not due to increased receptor number. Calcium flux imaging was utilized to further quantify stiffness dependent downstream signaling. To analyze VEGF calcium flux movies we developed a custom MATLAB software script that is able to individually identify cells, normalize each calcium signal value to each cell's individual background, and analyze the data. A variety of outputs are produced such as the magnitude of response, number of local maximums each cell experiences and clusters of coordinated cells. Utilizing this analysis tool, we were also able to show that endothelial cells on the softest substrates were most sensitive and coordinated in their response. Through various means we show that minor modifications in matrix binding of VEGF due to stiffness can dramatically drive endothelial response to VEGF. Supported by BrightFocus grant (M2012014).

N. GONORRHOEAE INDUCES LOCALIZATION OF THE INHIBITOR OF APOPTOSIS PROTEIN CIAP2 TO EXOSOMES

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The family of inhibitors of apoptosis proteins (IAPs) has been implicated in the establishment of microbial infection in host target cells and in cancer progression, due to their dual role in apoptosis and inflammation. It has been reported that survivin, an IAP family member, has both an intracellular and extracellular role in cancer cells by inhibiting apoptosis while promoting proliferative and metastatic potential in neighboring cells when secreted in exosomes. Exosomes are small lipid vesicles released from various cell types and deliver messages in the form of protein and mRNA to neighboring cells near and far. We have previously established that *Neisseria gonorrhoeae* protects against staurosporine-induced apoptosis in transformed human endocervical epithelial cells (End/E6E7 cells) as well as HeLa cells. The ability of *N. gonorrhoeae* to inhibit apoptosis correlates with the upregulation of cIAP2. In this study, we have further characterized the role of cIAP2 in the host inflammatory response to gonococcal infection. In End/E6E7 cells, we demonstrate that gonococcal infection induces a transient increase in intracellular cIAP2, and a stable increase in extracellular cIAP2. Extracellular cIAP2 localization is not due to membrane disruption or cell death during infection. Notably, extracellular cIAP2 is located in exosomes released after *N.gonorrhoeae* infection, supporting that cIAP2 is released in a controlled mechanism. Furthermore, *N. gonorrhoeae* significantly induces exosome production. Collectively, our studies reveal significant alterations in exosome production and cIAP2 expression following gonococcal infection. Such changes may affect both inflammation and apoptosis of infected epithelial cells and potentially, uninfected neighboring cells.

REGULATION OF AhR ACTIVATION IN TRIPLE NEGATIVE BREAST CANCER CELLS BY TRYPTOPHAN METABOLITES.

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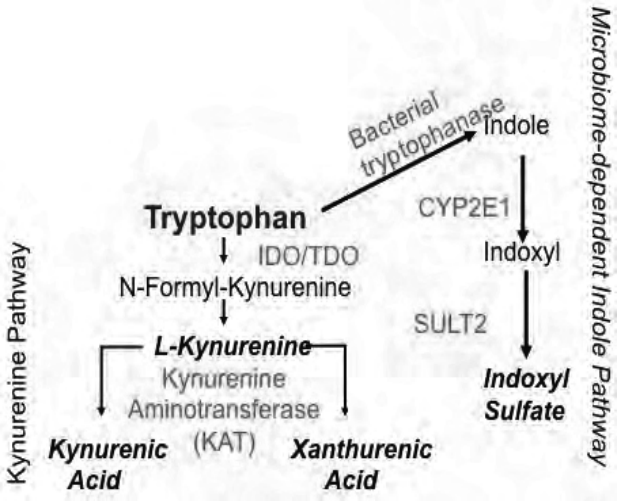
This study focuses on the AhR, an environmental chemical receptor/transcription factor long associated with cancer. Historically, AhR activation by environmental ligands was seen to facilitate mutations through CYP1 up-regulation. Our findings suggested that the AhR plays potentially a more critical role in the later, lethal stages of cancer. This new concept represents a major paradigm shift and begs the question of what endogenous ligands drive AhR activity and tumor invasion in the absence of environmental AhR ligands (constitutive AhR activity). Our long-term goal is to identify endogenous ligands present in human mammary tumor cells, to determine how their production is controlled, and to assess how environmental AhR ligands alter this process. Previous studies provide evidence that tryptophan metabolites are potent endogenous AhR ligands. Our data demonstrate that four tryptophan metabolites, kynurenine (KYN), kynurenic acid (KA), xanthurenic acid (XA), and indoxyl sulfate (IS), are potent AhR agonists. We therefore hypothesize that: Tryptophan metabolites produced by KYN and IS pathways of tryptophan metabolism (Figure 1) drive constitutive AhR activity and thereby enforce cell growth and/or invasion of malignant human mammary epithelial cells. As a corollary, we postulate that environmental AhR ligands distort this signaling. Treatment with metabolites of the IS and KYN pathway induced AhR activity and up-regulatd expression of AhR-driven genes in all mammary epithelial cell lines tested. Moreover IS disrupted normal colony formation of non-malignant mammary epithelial cells in Matrigel and lead to increased invasiveness of non-malignant cells in a trans-well assay. qPCR and western blot analysis revealed that malignant mammary epithelial cell lines highly express TDO, a key enzyme required for the KYN pathway, while moderate to no expression of TDO was found in non-malignant cells. Reduction of *TDO* expression using a short-hairpin TDO-directed RNA resulted in reduction of *Cyp1B1*, a well-known transcriptional target of the AhR. In addition, down-regulation of *TDO* lead to reduction in expression of metalloproteinases that play a role in cell invasion, *MMP1, MMP9, MMP3.* Previous microarray data and promoter analysis suggests that expression of these metalloproteinases in mammary epithelial cell lines is driven by transcriptional activity of the AhR. We hypothesize that reduced expression AhR target genes after shRNA-induced TDO down-regulation is due to reduced production of endogenous AhR ligands via the kynurenine pathway of tryptophan metabolism. LC-MS analysis is currently being used to identify and quantify putative AhR ligands produced by tryptophan metabolism in malignant and non-malignant mammary epithelial cells.

Figure 1: Tryptophan Metabolism



Typtophan is metabolized through the kynurenine pathway and, via the bacterial enzyme tryptophanase, the indole pathways. AhR lignds are in bold italics.

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MAST CELLS ARE A KEY SOURCE OF NEUROTROPHIN 4 (NT4) FOR ALLERGEN-INDUCED NEUROPLASTICITY IN NEONATAL ALLERGIC ASTHMA

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Asthma is a chronic inflammatory disease of the airway with a hallmark of hyper-reactive bronchial smooth muscle (BSM). In the United States alone, about 9 million children have asthma. Asthma in children is linked with exposure to pollutants, allergens or respiratory viral infection. Often, childhood asthma persists into adulthood. Our recent study in a mouse model of neonatal asthma demonstrates that early-life allergen exposure increases BSM innervation by up regulating levels of neurotrophin 4 (NT4). Neurotrophins are trophic factors for neurons that support axon outgrowth, survival and differentiation. We found that the hyper innervation of BSM is causally linked to persistent airway hyper-reactivity into adulthood. However, how allergen exposure increases NT4 is unknown. Here we show that the NT4-dependent increase in innervation is associated with inflammation and positively correlates with the abundance of mast cells in the airway. Mast cells are tissue specific cells that release diverse mediators during an allergic response. We observed that, mast cells express NT4. In addition, mast cell deficient pups (*Kit* $^{W\text{-}sh}$) do not exhibit any change in innervation after allergen exposure. Collectively, our data suggest that mast cells are a source of NT4 mediated BSM innervation. Future studies will further investigate mechanisms that regulate NT4 expression and release from mast cells in neonatal asthma model. The objective of our study is to unravel the mechanism involved in the complex crosstalk between inflammation, nerves and BSM leading to BSM remodeling. Our research findings will provide novel drug targets to improve asthma treatment and outcome.

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LOSS OF ROBO2 IN PODOCYTES PROTECTS ADULT MICE FROM ACUTE GLOMERULAR INJURY

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BACKGROUND: Robo2 is a receptor that plays an important role in embryonic kidney development. We have recently found that Robo2 is also expressed in adult mouse kidney glomerular podocytes. However, the role of Robo2 in adult mouse kidney is not clear. **METHODS**: To test the hypothesis that loss of Robo2 in glomerular podocytes can affect the outcome of acute glomerular injury, we performed two in vivo acute glomerular injury models (nephrotoxic serum (NTS) injection and protamine sulfate (PS) perfusion) in Robo2 podocyte specific knockout mice and their wildtype controls. Kidney glomerular structure was analyzed by histology and electron microscope after injury. Renal function was analyzed by measuring urine albumin and urine creatinine. **RESULTS**: We found that Robo2 podocyte specific knockout mice have lower albumin/ creatinine ratio compared to the wild-type controls 24 hours after NTS injury (p=3.61894E-05). By transmission electron microscopy, we found that Robo2 podocyte specific knockout mice have higher foot process density and more slit-diaphragm compared to the wild-type controls after either protamine sulfate injury or NTS injury, suggesting loss of Robo2 in podocytes alleviates foot process enfacement and protects mice from short term acute podocyte injury.
CONCLUSION: Robo2 plays an important role in adult mouse kidney. Loss of Robo2 protects adult mice from acute glomerular injury. The mechanism of Robo2 renoprotective role in adult mice remains to be determined.

DISTINCT LIPID A MOIETIES CONTRIBUTE TO PATHOGEN-INDUCED SITE-SPECIFIC VASCULAR INFLAMMATION

Connie Slocum, Stephen R. Coats, Ning Hua, Carolyn Kramer, George Papadopoulos, Ellen O. Weinberg, Cynthia V. Gudino, James A. Hamilton, Richard P. Darveau and Caroline A. Genco

Molecular and Translational Medicine

Several successful pathogens have evolved mechanisms to evade host defense, resulting in the establishment of persistent and chronic infections. One such pathogen, *Porphyromonas gingivalis*, is the primary etiological agent of periodontal disease and is associated with systemic inflammation manifested as atherosclerosis. *P. gingivalis* expresses an atypical LPS structure containing heterogeneous lipid A species, that exhibit functional specificity resulting in TLR4 agonist or antagonist activity, or are immunologically inert. In this study we observed production of antagonistic lipid A was associated with the induction of low levels of TLR4-dependent proinflammatory mediators, failed activation of the inflammasome and increased bacterial survival in macrophages. Oral infection of $ApoE^{-/-}$ mice with this strain resulted in vascular inflammation, macrophage accumulation and atherosclerosis progression. In contrast, a *P. gingivalis* strain producing agonistic lipid A augmented levels of proinflammatory mediators and activated the inflammasome, resulting in host cell lysis and decreased bacterial survival. $ApoE^{-/-}$ mice infected with this strain exhibited diminished vascular inflammation, macrophage accumulation, and atherosclerosis progression. Notably, the ability of *P. gingivalis* to induce local inflammatory bone loss was independent of lipid A expression, indicative of distinct mechanisms for induction of local versus systemic inflammation by this pathogen. Collectively, our results demonstrate that *P. gingivalis* escapes TLR4-mediated bacterial clearance in the host, facilitating chronic inflammation in the vasculature. These studies support the emerging concept that pathogen-mediated chronic inflammatory disorders result from specific pathogen-mediated evasion strategies resulting in low-grade chronic inflammation.

THE ARYL HYDROCARBON RECEPTOR CONTROLS BREAST CANCER STEM-LIKE CELL DEVELOPMENT AND FUNCTION.

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Molecular and Translational Medicine

In 1940, an American woman's risk of getting breast cancer was 1 in 14. Since then, the age-adjusted incidence has increased such that 1 in 8 women born this year will be diagnosed with breast cancer{DeSantis, 2011 #434@@hid{DeSantis, 2011 #434}den}. Analyses of known risk factors such as diet, exercise, and hormone replacement therapy do not completely explain the increase in breast cancer incidences. A meta-analysis of over 150 published studies on breast cancer and the environment implicates environmental chemicals, including aryl hydrocarbon receptor (AhR) ligands, in breast cancer risk. Tumor invasion and metastasis following relapse is the cause of death in nearly all breast cancer patients. The cancer stem cell (CSC) theory hypothesizes that cancers and their metastases are driven by breast cancer stem-like cells (BCS_LCs). BCS_LCs are defined by their activity and expression of aldehyde dehydrogenase (ALDH), expression of a set of genes associated with "stem-ness" and epithelial-to-mesenchymal transition (EMT), resistance to chemotherapeutics, and ability to self-renew. Recent studies implicate the AhR, a transcription factor involved in tumorigenesis, in the development and/or function of stem cells, which share properties with CSC. Therefore, we postulated that the AhR plays a role in the development or function of BCS_LCs. RHere, we present data demonstrating high levels of AhR in BCS_LCs from aggressive human ER^-, PR^-, $Her2^-$ breast cancers. The results indicate that AhR hyper-activation increases, and AHR inhibition with pharmacological or molecular agents decreases, expression of BCS_LC characteristics. Our findings further implicate the role of environmental chemicals in breast cancer risk through increasing breast cancer progression and survival after chemotherapy.

Graduate Program in Neuroscience

Participants

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*

DYNAMIC RECRUITMENT OF HUMAN FRONTAL LOBE NETWORKS FOR TEMPORAL AND SPATIAL PROCESSING

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Graduate Program for Neuroscience

While the sensory modalities of audition and vision both can both provide amodal spatial and temporal domain information, the quality and resolution of this amodal information is not equal across the senses. The visual system exhibits high spatial but low temporal resolution; conversely, the auditory system exhibits low spatial but high temporal resolution. We propose that tasks in one sensory modality may dynamically recruit the other modality's attention network when the other modality offers higher resolution in the relevant domain (e.g., visual-temporal or auditory-spatial tasks). We call this the Domain Recruitment Hypothesis. To test this hypothesis, we performed 3 sets of fMRI experiments. First, we employed a sustained attention task with competing auditory and visual stimuli to define sensory-biased cortical regions in both frontal and posterior areas. We then used resting-state functional connectivity to confirm regions with the same sensory-bias belong to visual or auditory attention networks. In the final experiment, we employed 4 short-term memory change detection tasks: visual-temporal, visual-spatial, auditory-temporal, and auditory-spatial. The auditory-spatial task (vs. the auditory-temporal task) significantly recruited the visual network. Similarly, the visual-temporal task (vs. the visual-spatial task) significantly recruited the auditory network. These observations support the Domain Recruitment Hypothesis. Our findings have important implications for understanding the functional organization of human cortex and the neural substrates of cognitive control. This work was supported by CELEST, a National Science Foundation Science of Learning Center, (NSF SMA-0835976) and the National Institutes of Health (NIH R01EY022229, 1F31MH101963).

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STRIATOPALLIDAL NEURONS CONTROL MOTOR ACTIVITY THROUGH STRIATAL COLLATERALS

Surpris Maripierre – Jiang-Fan Chen Lab

Graduate Program for Neuroscience

The basal ganglia are the principal input and output structure of the brain that is involved in motor function and motivational behaviors. The striatum is the main input structure of the basal ganglia receiving signals from the cortex and sending projections via the direct and indirect pathways. While the direct and indirect pathways MSNs is strictly segregated in term of their anatomical connection, projections and molecular expression, recent optogenetic and electrophysiological studies suggest that both the direct and indirect pathways are activated during motor initiation and they may play complementary roles in action initiation. In addition, neurochemical data combined with single cell recording emphasis the presence of profuse projection and collateralization within the basal ganglia circuit. To dissect out the role of the indirect pathway in the control of motor activity, we have developed a new transgenic mouse line expressing ChR2 or Arch-GFP selectively in the medium spiny neurons (MSN) of the indirect pathway under control of adenosine A_{2A} receptor (Adora2a) gene promoter. To decipher the functional role of axon collaterals and their possible effects on striatopallidal projections of the indirect pathway, we optogenetically stimulated and silenced the striatopallidal neurons in the dorsal striatum or the striatopallidal projections in globus pallidus (GP) in vivo. Our results indicate that light activation of ChR2 in the striatopallidal axon projections in GP increased locomotor activity in a frequency-dependent manner, with tics-like behavior emerging at 1 Hz and a dramatic increase in ambulatory movement occurring at 20 Hz. We validated the motor enhancing effect of stimulating the indirect pathway in GP of *Ai32 (ChR2) x Adora2a-Cre* mice by inhibiting the striatopallidal projections in the GP using *Ai35 (Arch) x Adora2a-Cre* mice (i.e. reducing striatopallidal inhibition of GPe). This resulted in predicted result of suppressing movement. Coupled with immediate early gene expression analysis we showed that light activation of ChR2 or Arch in the striatum selectively stimulates/silences both the striatopallidal neurons as well as its extensive striatal collaterals; activation of the axons in the GP selectively stimulates/silences only the presynaptic striatopallidal neurons. These results raise new questions regarding the complexity of the role of the indirect pathway in control of motor activity under normal conditions and shine new lights on debated issue about the net effect of the collaterals on striatal output of GP neurons.

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LOCAL AND DISTANT FUNCTIONAL CONNECTIVITY CHANGES VARY BASED ON FUNCTIONAL NETWORK IN MULTIPLE SCLEROSIS

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Graduate Program in Neuroscience

Local functional connectivity (LFC) and distant functional connectivity (DFC) metrics combine network theory and resting state functional connectivity MRI (fcMRI) to estimate regional degree variations across the cortex. The balance of LFC and DFC across the cortex is important for network efficiency and may serve as an early marker of pathology. Imaging data in 22 multiple sclerosis (MS) subjects and 19 age- and gender-matched healthy controls (HC) were acquired at 3T. LFC and DFC maps were generated in each subject by correlating the time course extracted from each voxel to all voxels contained within (LFC) and outside of (DFC) a 14mm radius sphere around that voxel. Average network LFC was increased in MS compared to HC in cortical areas represented by the sensorimotor ($p=0.017$) and visual networks ($p=0.013$) and decreased in areas represented by heteromodal association networks, including DMN ($p=0.009$), dorsal attention ($p=0.027$) and frontoparietal control networks ($p=0.010$). Average network DFC was increased in MS compared to HC in the somatosensory network ($p=0.038$) and decreased in the dorsal attention network ($p=0.045$). Increased LFC in the sensorimotor network significantly correlated with low EDSS in MS ($tau=0.36$, $p=0.029$). LFC in RRMS was increased in primary sensory networks and decreased in higher order association networks. Similar trends were demonstrated for DFC. Intrinsic connectivity changes varied in a network specific manner, suggesting differential effects of MS pathology in specific functional networks. Specifically, somatomotor LFC may represent a promising marker of disability in MS.

ADAPTIVE DECODING OF EYE MOVEMENTS WITH A SIMPLE RECURRENT ARTIFICIAL NEURAL NETWORK

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Graduate Program for Neuroscience

A primary concern for brain-machine interfaces (BMI) is the development of decoding algorithms that are able to adapt to neural activity changes, and reduce the need for daily calibration. We used a simple recurrent artificial neural network (SRN) to test the feasibility of adaptive online decoding of eye movements in a macaque during a 6-choice delayed saccade task. Three consecutive days of 80-500 Hz local field potential data were previously recorded from dorsolateral prefrontal cortex, the frontal eye field, and the supplementary eye field. Whole day training was performed separately on the 1st and 2nd days to create models of the SRN, which were then used on the 2nd and 3rd days, respectively, as bases for online learning. Two days (i.e. the 2nd and 3rd days) of adaptive online decoding were thereby simulated by updating the SRN models after each sequential trial of the test data. Initial simulated online performance of the SRN models were above chance levels (~65-80%), and late performance of the SRN models were qualitatively similar to the results obtained from linear discriminant analysis run online in a closed-loop setting (~75-80% correct). Asymptotic performance during simulated online learning was achieved within ~100 trials—significantly fewer than the 600 training trials required for equivalent performance with the linear discriminant model. Our results indicate that a SRN can be used for online adaptive decoding without the need of calibration, and achieve a performance level comparable to non-adaptive decoders that are calibrated daily.

PATHOLOGICAL CHANGES IN RNA BINDING PROTEINS IN PARKINSON'S DISEASE

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Stress granule (SG) formation has emerged in the field of neurodegeneration as a compelling mechanism tightly correlated with diseased states. This study outlines the pathophysiology of neuronal changes in RNA binding proteins (RBPs) in Parkinson's disease (PD), and the potential for novel markers and therapeutic targets in this spectrum of diseases. Previous work in our lab has expounded on the importance of the RNA-binding protein TIA-1 in the misfolding and aggregation of tau protein, a major pathological species in Alzheimer's Disease. Here, we examined cingulate cortex from subjects with PD, Dementia with Lewy Bodies (DLB), Parkinson's Disease with Dementia (PDD), and control patients. Our data shows that TIA-1 exhibits changes in neurons throughout the cingulate cortex of PD, DLB and PDD subjects. Tissue sections were double stained for α-synuclein (α-syn) along side a number of stress granule markers (TIA-1, TTP, HUD, G3BP, etc.). Analysis of TIA-1 in diseased brains shows movement of TIA-1 from the nucleus to the cytoplasm throughout cortical neurons. Other changes included a strong increase in TIA-1 movement in DLB brains when compared to PD and PDD. This study highlights the potential importance of stress granule formation in PD, and points to putative pathophysiological changes in PD and PD-like diseased brains that could serve as early detectors in the race to combat degeneration.

Graduate Program in Nutrition & Metabolism

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BENEFICIAL EFFECTS OF DIETARY POTASSIUM AND POTASSIUM:SODIUM RATIO ON ADOLESCENT BLOOD PRESSURE

Buendia, Justin Rene; Bradlee, M. Loring; Moore, Lynn; Singer, Martha

Childhood blood pressure (BP) is an important predictor of adult blood pressure. Identification of modifiable risk factors for elevated blood pressure levels during adolescence has the potential to reduce the risk of adult hypertension. Longitudinal data on the effects of dietary sodium and potassium on adolescent BP are lacking.

The objective of the study was to examine the effects of dietary potassium, sodium, and the potassium:sodium ratio (K:Na) throughout adolescence on blood pressure at the end of that period and to determine whether these effects are modified by other dietary factors.

Data from 2330 girls, initially ages 9-10, and followed for 10 years in the National Growth and Health Study, were used to address these questions.

Mean potassium and sodium intakes were 3.1 and 2.0 g, respectively, from an average of 6.6 sets of 3-day diet records.

Analysis of covariance (ANCOVA) models were used to estimate mean systolic (SBP) and diastolic (DBP) blood pressures at 18-20 yrs of age, controlling for height, race, TV/video viewing time, physical activity, total energy intake, and BMI.

Girls in the highest tertile of potassium intake (vs. lowest) had the lowest adjusted mean SBP (108.46 vs. 109.43 mm Hg; p-trend=0.030) and DBP (65.18 vs. 65.94 mm Hg; p-trend=0.073) at the end of follow up. Similar results were observed for the K:Na ratio (SBP 108.07 vs. 109.50 mm Hg; p-trend=0.021; DBP 65.02 vs. 65.85; p-trend=0.151). Finally, mineral intake as a part of a healthy eating pattern was examined.

In this analysis, girls with a higher K:Na ratio (≥0.7) combined with high intakes of total fruit and vegetable (FV), fiber, or dairy had SBP levels that were approximately 2 mm Hg lower at the end of follow up than those with lower intake ($p<0.05$ for all diet patterns). Adolescents with a higher K:Na ratio with higher total FV or fiber intakes also had the lowest DBP levels at the end of follow up.

Dietary sodium intake was generally unrelated to BP levels regardless of other diet patterns.

Adolescent girls who consumed a high potassium and K:Na alone and particularly in the context of healthy eating patterns throughout adolescence had lower SBP and DBP levels at the end of adolescence.

This study suggests that dietary potassium may play a more important role than sodium in determining adolescent BP levels. Thus, increasing dietary potassium intakes during childhood may reduce the risk of hypertension later in life.

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) AND LIRAGLUTIDE, A SYNTHETIC GLP-1 ANALOG, INHIBIT INFLAMMATION IN HUMAN AORTIC ENDOTHELIAL CELLS VIA CALCIUM AND AMPK DEPENDENT MECHANISMS

Nadia Krasner

Glucagon-like peptide-1 (GLP-1) synthetic analog therapies are prescribed for type 2 diabetes due to their effects on glycemic control. Recent studies also suggest that they may have cardiovascular benefits; however, the mechanism responsible for this is unknown. To examine this, we evaluated the effects of GLP-1 (30pM) and GLP-1 synthetic analog, liraglutide (100nM), on human aortic endothelial cells (HAECs). We found that incubation with GLP-1 or liraglutide stimulates an immediate increase in intracellular calcium, which activates calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ). This led to a rapid 2.5 fold increase in the phosphorylation of AMP-activated protein kinase (AMPK) and calcium/calmodulin-dependent protein kinase I (CaMK1). In addition, both caused a 2-fold increase in the phosphorylation of AMPK/CaMK1 targets: eNOS and CREB. Inhibition of CaMKKβ with STO-609 (0.5ug/mL) blocked phosphorylation of AMPK and CaMK1. Incubation of HAECs for three hours with lipopolysaccharide (LPS, 2ug/mL) or TNFα (10ng/mL) increased expression of vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, and subsequent THP-1 monocyte adhesion, an initiating event in atherogenesis. Pre-incubation with either GLP-1 or liraglutide inhibited these events. Furthermore, pre-incubation with the CaMKK inhibitor STO-609, or use of lentivirus shRNA to knock down AMPK, blocked the inhibitory effects of both GLP-1 and liraglutide on monocyte adhesion. These results suggest that both have an endothelial cell specific effect which may contribute to the improved CVD risk in patients on synthetic agonist therapies.

PERI-DROPLET MITOCHONDRIA IN BROWN ADIPOCYTES FORM AN EXCLUSIVE SUBPOPULATION OF MITOCHONDRIA

Mahdaviani Kiana[1], Chess David[1], Liesa Marc[1], Shirihai Orian S[1], Trudeau Kyle[1], Twig Gilad[3], Wikstrom Jakob D[1,2.] Nutrition and Metabolism Program

Background and aims: Mitochondria play essential roles in brown adipose tissue differentiation and function. We have recently shown that mitochondrial dynamics is a physiological regulator of adrenergically-induced changes in energy expenditure. Moreover, we and others have found that changes in mitochondrial architecture may represent adaptive changes to bioenergetics needs. Conditions requiring high mitochondrial ATP synthesis capacity and/or efficiency, such as limited nutrient availability, are associated with mitochondrial elongation. Whereas, conditions of excess energy supply and low ATP demand acutely induce mitochondrial fragmentation. However it remains unclear if diversity in mitochondrial architecture within the individual cell may play a role in generating functional specialization of subpopulations of mitochondria within the cell. We rationalized that the brown adipocyte may shed light on this question as its mitochondria are required for various and competing tasks, such as lipogenesis and beta oxidation as well as ATP synthesis and uncoupling, raising the possibility that the brown adipocyte may harbor a diverse set of mitochondria which are structured to fit different functions.

Materials and methods: Brown pre-adipocytes were harvested from 3-week-old wild-type male C57BL6/J mice and differentiated in culture. Mitochondrial membrane potential, motility and morphology were measured using TMRE and PAGFP respectively with Zeiss LSM 710 confocal microscope. NADH autofluorescent was used to measure the NADH content of the mitochondria. Protein import and turnover were measured using MitoTimer probe.

Results: Mitochondria in brown adipocyte are divided to two different populations, mitochondria that are found with submicron proximity to the lipid droplet or Peri-droplet (PD) mitochondria, and mitochondria that are located at least 5μm away from the vicinity of a lipid droplet or Cytoplasmic (C)mitochondria. Photo-conversion of matrix targeted photoactivatable GFP shows that these two populations rarely mix and that mitochondria remain faithful to the lipid droplet they adhere to and do not share their matrix content with other mitochondria, nor do they switch their affiliation from one group to another. These studies also show that PD-mitochondria are more elongated while the C- mitochondria tend to be smaller in size suggesting a more coupled respiratory function and involvement in ATP synthesis. In addition, using TMRE, we found that PD- mitochondria in brown adipocytes but not in other lipid-containing cells are in different energetic state than C-mitochondria. Ratiometric imaging of membrane potential and NADH/NAD ratio show that PD-Mitochondria have higher NADH/NAD ratio and more polarized membrane potential. Protein import and turnover studies using MitoTimer also indicate that PD- mitochondria have a higher rate of protein import and biogenesis, in agreement with the hyperpolarized state. Altogether these observations support a hypothesis that PD-mitochondria have a higher TCA cycle activity.

Conclusion: These data suggests that peridroplet mitochondria represent a functionally exclusive subpopulation of mitochondria in terms of biogenesis and function. This may also demonstrates that the unique architectural characteristics of cells in diverse bioenergetics states may apply subcellularily to a diverse set of mitochondria within the cell, fulfilling different metabolic functions.

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VITAMIN D AND PNEUMONIA IN CHILDREN IN THE HIGH ECUADORIAN ANDES

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Background: Vitamin D is involved in the regulation of lymphocytes, neutrophils, macrophages, and respiratory epithelial cells. Given this immunomodulatory role, recent studies are exploring the role that vitamin D deficiency may play with acute lower respiratory infections (ALRI) plays.

Objective: To determine the association between vitamin D status and pneumonia in children aged 6-36 months in the Ecuadorian Andes.

Design: We conducted a secondary analysis of a randomized controlled trial, the Vitamin A and Zinc Prevention of Pneumonia (VAZPOP) study, a community-based study involving children aged 6 to 36 months living in urban slums of Quito, Ecuador who were randomized to receive vitamin A and/or zinc and followed for 48 weeks to determine the impact on pneumonia incidence. ALRI was defined as an increased respiratory rate of 40 breaths/minute and/or lower respiratory secretions with at least a cough, fever, or chest retractions. Vitamin D insufficiency was defined as a serum 25(OH)D concentration of 20-29 ng/mL and deficiency as <20 ng/mL. Serum 25(OH)D levels were assayed using an automated immunoassay system (Immunodiagnostics Systems).

Results: Of the 494 subjects who had 25(OH)D concentrations measured, 67 (14%) incident cases of pneumonia occurred over the course of 48 weeks of follow-up. After controlling for treatment arm, vitamin D insufficient children were more likely to develop pneumonia than those who were vitamin D sufficient (RR = 1.51; 95%CI = 0.84–2.72, p=0.17).

Conclusions: In Andean children, we found a non-significant trend towards an association between vitamin D insufficiency and ALRI.

Program in Oral Biology

Participants

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*

THE EFFECT OF SERUM ON IN VIVO EARLY MICROBIAL COLONIZATION OF TOOTH ENAMEL.

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Oral Biology

The oral environment surrounding the oral soft and hard tissues is determined by the biochemistry of saliva. Therefore, all molecular events occurring on exposed oral surfaces are influenced by the composition of this oral fluid. A particular exception applies to the space immediately above the gingival sulcus facing the tooth surface. This is due to the presence of gingival fluid, a well known entity in oral biology characterized by the slow outflow of a serum-like fluid. Once emanated from the gingival sulcus, this fluid mixes with saliva, creating a serum-saliva gradient. The gradient originates at the orifice of the gingival sulcus and becomes completely salivary in nature at some distance from the sulcus. It is well established that the biofilm of tooth surfaces is the major culprit for caries and periodontal disease development. The critical early event of biofilm formation is the specific interaction of microorganisms with the acquired enamel pellicle, a thin protein film coating tooth enamel. This pellicle structure dictates the specific adsorption of biofilm microorganisms. We hypothesize that the microbial binding specificity may change depending on the type of adsorbed proteins present being derived either from serum or saliva.

Objective: To investigate the effect of serum proteins binding to enamel surfaces on the adsorption of the early microbial colonizers.

Methods: 5 mL of blood were obtained by venipuncture and its serum fraction prepared. Pellicle and dental biofilm were removed from the buccal surfaces of teeth comprising first molar to first molar in both arches. Two- thirds of the cleaned coronal surfaces of incisor, canines and pre-molars of the upper and lower right arch were coated with 5 μ L of serum derived form the same subject. As a negative control, the incisors, canines and pre-molars of the upper and lower left arch were coated with 5 μ L of water. The teeth were exposed to the normal oral environment for a specified period with the subject refraining from the intake of food or drinks, except water. After exposures to oral conditions for 0, 2, 4 and 6 hours biofilm material was harvested and its microbial composition analyzed by the Human Oral Microbial Identification Microarray.

Preliminary Results: Adequate coverage of the enamel surfaces was achieved with 5 μ L of serum. Microbial DNA was successfully prepared from the collected sample of a single subject. The intended enrollment for this study will be 10 subjects. Microbial composition data of serum and water covered tooth surfaces will be subjected to the paired Wilcoxon Signed-Rank Test and Spearman Correlation Coefficient analysis. Significance will be set at $p > 0.05$.

Conclusion: This will be the first study to investigate whether serum proteins in the in vivo formed acquired enamel pellicle will lead to biofilm characteristics conducive to periodontal disease development. The data could also provide new information on prevention and therapeutic management of the disease.

CILIARY COLOCALIZATION AND INTERACTION OF EVC &EVC2 PROTEINS

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Objectives: Ellis-van Creveld syndrome (EvC) is an autosomal recessive skeletal dysplasia, characterized by short ribs, short limbs, postaxial polydactyly, hyperplastic frena with shallow labial sulcus, peg shaped teeth, delayed eruption of teeth and dysplastic nails. Mutations in either of the genes *EVC* or *EVC2* lead to the manifestation of the same clinical phenotypes identified as EvC syndrome, though there is no significant sequence homology between them at the DNA or protein level. As patients with EvC syndrome have many dental abnormalities and previous reports demonstrated that Evc and Evc2 proteins localize at primary cilia, we hypothesize that ciliary colocalization of Evc and Evc2 proteins might play a role in the functions of these respective proteins.

Methods: The expression of Evc and Evc2 proteins was investigated by the Immunohistochemical staining method using mouse craniofacial tissues. Immunofluorescent staining was performed to determine cellular localization of endogenous Evc and Evc2 proteins in mammalian cells. The binding of Evc to Evc2 and the effect of Evc overexpression on Evc2 protein were also studied.

Results: Immunohistochemical staining demonstrated the expression of Evc and Evc2 proteins in ectodermal derivatives of mouse craniofacial tissue. Both Evc and Evc2 proteins were colocalized at cilia by Immunofluorescent staining. The binding assay confirmed that Evc and Evc2 proteins interact with each other and Evc overexpression appeared to modify the immunoreactive bands to Evc2.

Conclusion: Our data demonstrate that both Evc and Evc2 proteins are expressed in ectodermal derivatives including tooth and that both these proteins colocalize at cilia. Our data also show that Evc and Evc2 proteins interact with each other and that Evc appears to affect Evc2 protein modification which might play a crucial role in Evc/Evc2 protein complex.

Department of Pathology & Laboratory Medicine

Participants

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*

ACTIVATED PROTEIN C RELIEVES ER STRESS BUT NOT MORTALITY IN MOUSE KIDNEY INJURY

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Department of Pathology and Laboratory Medicine

Introduction: Endoplasmic reticulum (ER) stress-induced cell and organ injury accompanies inflammatory challenge and is relieved by activated protein C (APC), which is anti-coagulant and cytoprotective. Shiga toxin-2 (Stx2) from food-borne enterohemorrhagic *E.coli* causes potentially lethal hemolytic uremic syndrome with acute kidney injury (AKI) in severe infections. Stx2 is a ribotoxin that causes renal tubular injury with AKI in mice. This is complicated by glomerular thrombi in patients and nonhuman primate (*Papio)* models. Therapy options are limited by lack of early injury biomarkers.

Objective: We hypothesized that APC would be protective and ER stress molecules would report Stx2-induced renal injury. Experiments were designed to take advantage of differential responses to Stx2 in mice (renal tubular AKI) and baboons (coagulopathy and AKI).

Methods: Mice were challenged with lethal Stx2 (1ng i.p; days 0,3) +/- APC (20 ug i.p; days 0-3) or saline with periodic phlebotomy. Kidney mRNA for qPCR was harvested at early euthanasia (day 2-3) or at endpoint ($n \geq 5$/group). Baboons received lethal Stx2 (50 ng/kg) +/- APC (2.4 mg/kg) at 24 or 48 hours after challenge and pathophysiology was monitored by established methods.

Results: Stx2 challenged mice developed terminal AKI with elevated BUN (Stx2, 81.0±15.7 *vs* saline, 31.3±10.4 mg/dL, $p<0.001$) and kidney AKI markers NGAL (37.3-fold; $p<0.01$) and KIM1 (69.5-fold; $p<0.001$). On day 2, ER stress markers HSP40 and spliced XBP1 mRNA were increased (2.8±0.6-fold,$p<0.01$; 1.79±0.72-fold, $p<0.05$) and on day 3, ER stress marker CHOP increased 8.74-fold ($p<0.01$). At endpoint, kidney anti-apoptotic Bcl2 and pro-apoptotic DR5 mRNA were decreased (0.44±0.18-fold, $p<0.001$) and increased (6.38±3.71-fold, $p<0.01$), respectively. APC co-treatment of Stx2 mice delayed rising BUN ($p<0.05$) and abrogated kidney CHOP mRNA (0.32±0.17-fold, $p<0.001$), but did not alter mortality (5.1±1.1 *vs* 5.6±0.5 days). In contrast, baboons were rescued from lethal Stx2 by delayed APC treatment, accompanied by significantly delayed BUN and creatinine increases.

Conclusions: This study identifies ER stress responses to be early indicators of Stx2-induced cell injury *in vivo* and possibly of value as early biomarkers. Reduced ER stress by APC did not associate with improved mortality in the mouse model, but such treatment was successful in the more complex baboon model.

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PODOCYTE SPECIFIC DELETION OF MYH9 ALTERS CYTOSKELETAL DYNAMICS AND PODOCYTE FUNCTION IN VITRO, AND EXAGGERATES GLOMERULAR DISEASE IN DOCA-SALT HYPERTENSION.

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Single nucleotide polymorphisms present in the gene MYH9 encoding the protein non-muscle myosin heavy chain IIA (NMHCIIA), have been found to be associated with increased incidence of chronic kidney disease. Glomerular capillary hypertension is a pervasive feature of progressive chronic kidney disease (CKD), and the podocytes have been shown to be susceptible to injury when glomerular capillary hypertension is present. In this study we seek to investigate the role of Myh9 in podocytes in vitro, and in the presence of increased glomerular capillary hypertension in vivo.

Adult C57BL/6 podocyte-specific Myh9 knockout (KO) mice and controls were subjected to uninephrectomy and deoxycorticosterone acetate (DOCA)-salt treatment for 6 weeks with both control and podocyte Myh9 KO mice showing increased systemic blood pressure 1 week after DOCA-salt treatment. Podocyte Myh9 KO mice developed widespread juxtamedullary glomerulosclerosis, segmental cellular proliferation within glomeruli, protein casts and mild glomerular ultrastructural changes in normal-appearing glomeruli. Proteinuria was markedly present in DOCA-salt treated podocyte Myh9 KO mice as compared to DOCA-salt treated control mice. In vitro, knockdown of podocyte Myh9 resulted in changes in podocyte morphology, altered cell cytoskeletal structure, reduced cell traction, increased cell permeability to labelled dextrans and increased cell motility compared to podocytes with Myh9.

In summary, Myh9 deficiency in podocytes in C57BL6 mice is associated with increased susceptibility to glomerulopathy in a DOCA-salt uninephrectomy model. NMHCIIA is required for the regulation of podocyte cytoskeletal structure, traction, permeability and motility. Myh9 is therefore necessary for podocyte function against glomerular damage induced by hypertension.

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THE HIPPO PATHWAY EFFECTOR YAP CONTROLS PATTERNING AND DIFFERENTIATION OF THE AIRWAY EPITHELIAL PROGENITORS

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The mechanisms by which epithelial progenitor cells integrate local signals to balance expansion with differentiation and regulate patterning during organogenesis are still poorly understood. Here we provide evidence of a novel regulatory mechanism implicating the Hippo pathway effector Yap in progenitor cell specification and patterning of the developing lung. We demonstrate that when epithelial tubules are forming and branching, a nucleocytoplasmic shift of Yap marks the boundary between the progenitors of the distal lung and the airway compartment. Remarkably, at this transition zone, Yap specifies a transcriptional program that controls the expression of Sox2, initiating an airway progenitor cell program key to generate the airway epithelium and its branched tubular structures. In Yap deficient mice, epithelial progenitors are unable to properly respond to local Tgf-beta-induced cues to control levels and distribution of Sox2, resulting in inability to form airways. We found that Yap levels and nuclear-cytoplasmic localization strongly influenced the differentiation status of airway progenitors later in development and in adult life. Our data reveals a crucial role for Yap in specification and differentiation of airway progenitors likely to be also relevant in regeneration-repair of the adult epithelium in pathological conditions.

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NEISSERIA GONORRHOEAE MODULATES IMMUNE CELL SURVIVAL THROUGH PYROPTOSIS

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Pathology and Laboratory Medicine

Cell death is a common mechanism for maintaining immune cell homeostasis and is induced in response to both endogenous and exogenous stimuli. Classically, cell death occurs through either apoptosis or necrosis. However, recent studies have defined additional cell death pathways including pyroptosis and pyronecrosis. In macrophages exogenous stimulation with some Gram-negative bacteria has been demonstrated to induce pyroptosis. Due to inflammasome activation and the spilling of intracellular components pyroptosis is highly pro-inflammatory. Recent studies have reported that the Gram-negative pathogen *Neisseria gonorrhoeae* induces pyronecrotic cell death in monocytic cells. In contrast, other immune cells, such as phagocytic neutrophils, are resistant to cell death following stimulation with *N. gonorrhoeae*. We have previously shown *N. gonorrhoeae* inhibits apoptosis in human endocervical cells and results in increased levels of cIAP2, an inhibitor of apoptosis protein, both intracellularly and extracellularly. In this study we have further characterized the cell death pathways induced by *N. gonorrhoeae* in macrophages. We demonstrate that stimulation of macrophages with *N. gonorrhoeae* induces cell death in a lytic manner. *N. gonorrhoeae* stimulation did not activate caspase-3, but did activate caspase-1. We conclude that *N. gonorrhoeae* stimulates cell death in human macrophages by pyroptosis and postulate that this contributes to both bacterial persistence and the induction of inflammatory pathways.

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SEROTYPE-MISMATCHED PNEUMOCOCCAL INFECTIONS ESTABLISH HETEROTYPIC IMMUNE PROTECTION AGAINST PNEUMONIA

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The adaptive immune responses normally protecting the lung against diverse serotypes of pneumococcus remain unclear. We hypothesize that respiratory tract exposures to *S. pneumoniae* of unrelated serotypes establish heterotypic immunity in the lung that reduces susceptibility to subsequent pneumococcal pneumonia. We have established a unique mouse model in which mice are infected with diverse pneumococci in the respiratory tract, given time to recover, and then challenged with a very virulent serotype 3 pneumococcus (Sp3) in the deep lung, which they cannot typically eliminate. Prior exposures to unrelated pneumococci proved to be highly protective, with multi-log reductions in bacterial burdens in the lungs of mice which were previously exposed to unrelated pneumococci, compared to vehicle controls. Thus, respiratory pneumococcal infections establish protective heterotypic immunity. We observed an accumulation of Th17 cells, which can mediate antibacterial pulmonary immunity, in the lungs of mice after pneumococcal exposure. Significantly increased Th17 cells also accumulated in the lungs of mice during heterotypic protection against Sp3 pneumonia, and were associated with reduced bacterial burden. Capsule-independent anti-pneumococcal antibodies are additional potential effector mechanisms. Serial respiratory exposures to unrelated pneumococcal serotypes resulted in plasma IgG that could bind to the surface of Sp3, which presumably was not polysaccharide capsule-specific. Contrasting with plasma from vehicle-exposed mice, plasma from mice previously exposed to unrelated pneumococci was sufficient to protect naïve mice against Sp3 pneumonia when transferred during an intratracheal infection. These data reveal that heterotypic immunity is generated by pneumococcal respiratory infections and protects against subsequent pneumonia. Mechanisms of protection involve a combination of pneumococcus-specific but serotype-independent Th17 cells and antibodies.

Department of Pharmacology & Experimental Therapeutics

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*

REGULATED PROTEIN AGGREGATION OF TIA1 INCREASES TAU AGGREGATION AND NEUROTOXICITY IN VITRO

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The eukaryotic stress response involves translational suppression of non-housekeeping proteins, and the sequestration of unnecessary mRNA transcripts into stress granules (SGs). This process is dependent on mRNA binding proteins (RBPs), such as T-cell intracellular antigen (TIA1), that interact with unnecessary mRNA transcripts through prion and poly-glycine like domains, and their aggregation mirrors that of proteins linked to neurodegenerative diseases. Recent advances in molecular genetics highlight the potential importance of SG biology in disease by associating multiple RBPs linked to SGs with neurodegenerative disease. The major difference between SG proteins and aggregation prone proteins linked to neurodegenerative diseases is that aggregation of SGs is transient and rapidly reverses when the stress is removed, while aggregates associated with disease are stable and accumulate over time. Our lab has identified SGs as a novel pathology in Alzheimer's disease. Our data suggests that TIA1 is intimately linked to tau pathogenesis, with TIA1 as a modifier of tau aggregation and associated toxicity. We find that TIA1 is present in a protein complex with total tau protein as well as pathological PHF1+ tau. Our in vitro data suggests that the expression of TIA1 increases phospho and conformational tau inclusion formation, while the expression of WT or P301L mutant tau is able to increase the formation and size of TIA1+ granules. We find that co-expression (but not individual expression) of TIA1 and tau leads to dendrite shortening in primary hippocampal neurons from Tau deficient mice, suggesting that an interaction between TIA1 and tau results in neurotoxicity. Our data suggests that the aggregation of tau may proceed through the SG pathway, with SG formation accelerating the pathophysiology of tau aggregation. Alternatively, the highly insoluble tau aggregates seen in disease could also serve as a nidus for further accelerated aggregation of SGs, leading to long-lived pathological SG formation. This over-active SG formation could act to sequester functional RBPs and/or interfere with mRNA transport and synthesis, each of which might potentiate neurodegeneration.

POST-TRANSCRIPTIONAL REGULATION OF A-SYNUCLEIN BY LRRK2 IN NEURODEGENERATIVE DISEASES

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In this study, we hypothesize that the expression and toxicity of α-synuclein are regulated by LRRK2 post-transcriptionally. Our preliminary data shows that co-expression of α-synuclein with either long or short 3' untranslated region (UTR) and wild-type LRRK2 in HEK293FT cells has a significant increase in α-synuclein expression in relative to without LRRK2 as measured by florescence level of the GFP-tagged α-synuclein. Further investigation of the effect of different mutant variants of LRRK2 (G2019S, R1441C) on α-synuclein expressions showed that both G2019S and R1441C significantly increased the level of α-synuclein expressions when it was co-transfected with the short 3'UTR isoform of α-synuclein transcript. However, only G2019S but not R1441C significantly increased the level of α-synuclein expressions when it was co-transfected with the long 3'UTR isoform of α-synuclein transcript. While both LRRK2 WT and G2019S significantly increase α-synuclein expressions in both the short and long 3'UTR, in conditions with the short 3'UTR, there is a significantly greater increase compare to the conditions with the long 3'UTR, which is in contrary to some other studies where long 3'UTR transcript seems to accumulate more and seems to be more toxic. In addition, our data shows that in rat primary culture cortical neurons, with co-transfection of LRRK2 or its mutant variants and α-synuclein with either long or short 3'UTR, there is a distinct difference in the accumulation and localization of α-synuclein in neurons as well as the morphology of neurons. In the presence of LRRK2, there is a higher level of α-synuclein protein expression in conditions with short 3'UTR compare to long 3'UTR. And in conditions with the short 3'UTR, neurons demonstrate longer processes, along with a greater number of α-synuclein containing puncta along the processes. This data suggests a differential effect of LRRK2 on α-synuclein with either long or short 3'UTR's expression and localization.

IMPAIRED PVN NEURONAL ACTIVITY IN RESPONSE TO ACUTE SODIUM CHALLENGE DRIVES PERSISTENT ELEVATIONS IN MAP IN CONSCIOUS RATS LACKING CNS Gαi$_2$ PROTEINS

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We have reported CNS Gαi2 proteins mediate the sympathoinhibitory responses to chronic high salt-intake. These studies assessed the role of Gαi2 proteins in the activity of PVN neurons in response to acute sodium challenge. 24-h ICV Gαi2 or SCR oligodeoxynucleotide (ODN; 25µg/5µl)-pretreated conscious rats were monitored for changes in MAP in response to IV bolus NaCl (3M; 0.14 ml/100g). Separate groups of rats were sacrificed at the peak MAP change (40 min post-IV NaCl) and Fos IHC was performed (N=6/gp). No difference was observed in sodium-evoked peak change in MAP between treatment groups (IV 3M NaCl ΔMAP [mmHg] SCR: 13±4 vs. Gαi2: 10±3), however MAP returned to control levels by 100 min in SCR but not Gαi2 treated rats (MAP 100min post-IV NaCl [mmHg] SCR: 134±2 vs. Gαi2: 146±3, $P<0.05$). In SCR, but not Gαi2, treated rats, an IV NaCl bolus produced significant reduction in Fos immunoreactivity ([PVN Fos positive cells] SCR control: 95±9 vs. post NaCl: 5±1, $p<0.05$, Gαi2 control 90±13: vs. post NaCl: 66±9). These data reveal a novel role of CNS Gαi2 proteins in the regulation of the neuronal response within the hypothalamic PVN to an acute sodium challenge that significantly elevates MAP. The sodium-induced decrease in Fos-positive PVN cells in SCR ODN rats likely reflects inhibition of neuronal activity facilitating the return of MAP to basal levels. In Gαi2 pretreated rats, failure to inhibit PVN neuronal activity represents a neural mechanism by which impairment of Gαi2 signal-transduction pathways may contribute to the pathophysiology of salt-sensitive hypertension.

MAPPING THE ENGRAILED 2 TRANSCRIPTOME IN THE GANGLIONIC EMINENCE OF THE DEVELOPING MAMMALIAN NERVOUS SYSTEM

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Pharmacology

Autism Spectrum Disorder (ASD) is characterized by repetitive behaviors and impairments in communication and socialization. Accumulating evidence suggests that developmental dysfunction of γ-aminobutyric acid (GABA) neurotransmission is a major factor in the etiology of ASDs. In early brain development, GABA is an excitatory neurotransmitter that drives synaptogenesis and orchestrates neural network formation. GABA signaling regulates proliferation, migration, and differentiation of neural progenitors. Evidence suggests that altered type A GABA receptor (GABAR) composition may contribute directly to disease pathogenesis. More specifically, single nucleotide polymorphisms (SNPs) in the human GABR alpha 4 and beta 1 subunit genes (GABRα4 and GABRβ1) are significantly associated with ASD in over 450 families. Our laboratory identified that these SNPs generate consensus sites for the engrailed 2 homeobox protein (EN2). EN2, an established ASD candidate gene, is a transcription factor critical for the development of the midbrain and cerebellum, brain structures with alterations in autistic patients. To determine the role of EN2 in GABAergic interneuron fate, we delivered EN2-specific shRNAs to the ganglionic eminence (GE) using in utero electroporation (IUE). Interestingly, our results demonstrate that EN2 may be necessary for progenitor cells to remain in the ventricular zone (VZ), which we hypothesize may be mediated through the regulation of GABR subunit gene expression. Transcriptome profiling of the GE VZ using RNAseq is being performed to identify targets of EN2 in the developing nervous system. These studies are the first of their kind to examine in vivo whether there is a potential relationship between EN2 and GABAergic neurotransmission.

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EVALUATION OF TISSUE SECTION CRYOSTORAGE ON IMMUNOHISTOCHEMISTRY

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Storage of tissue sections for significant periods is often required to allow tissue from multiple samples, acquired over months to years, to be processed together, in the same reagents, for quantitative studies using immunohistochemistry. Several protocols for storage in sucrose with different additives have been reported that assert there is no effect on immunogenicity. However, there have been no quantitative assessments of how long-term frozen storage in any cryoprotectant affects immunogenicity. The present study analyzed existing datasets, derived from monkey brain sections stored at -80° C in 15% phosphate buffered glycerol to determine whether time frozen in this cryoprotectant affected stereology counts of cells immunostained for NeuN, Parvalbumin, Orexin-A, Doublecortin, BrdU or pro-BDNF. Multiple regression analyses controlling for age and sex of the subjects in the different data sets showed that time in 15% glycerol did not affect stereological counts for any marker. Experiments are underway to compare our 15% glycerol cryoprotectant to four 30% sucrose based cryoprotectants for preservation of immunogenicity after frozen storage. Research Support: NIH grants R01-AG043640 and R01-AG042512

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A ROLE FOR CASEIN KINASE 1-EPSILON IN THE MOTIVATIONAL PROPERTIES OF OPIOIDS

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Pharmacology and Experimental Therapeutics

Recent genetic and pharmacological studies indicate that Casein kinase-1 (CK1) contributes to the behavioral properties of diverse drug classes, including psychostimulants, opioids, and ethanol. We previously found that selective pharmacological inhibition of casein kinase-1 epsilon (Csnk1e) enhanced the locomotor stimulant properties of the selective mu opioid receptor agonist fentanyl, indicating a negative regulatory role for Csnk1e in drug-induced behavioral responses. Here, we tested the hypothesis that *Csnk1e* negatively regulates the motivational properties of fentanyl. Using the conditioned place preference (CPP) assay, 24 hours post-assessment of initial preference for the drug-paired side on Day 1, mice received fentanyl (0-0.2 mg/kg, i.p.) on Days 2 and 4 and were confined to the drug-paired side, and saline (i.p.) on Days 3 and 5 and were confined to the other side. On Day 8, mice were assessed for fentanyl CPP (Day 8-Day 1). *Csnk1e* knockout mice showed a leftward shift in the inverted u-shaped curve for opioid reward, exhibiting enhanced reward at lower doses (0.05 mg/kg, i.p.) and significantly decreased reward at higher doses (0.2 mg/kg, i.p.). No differences were observed in fentanyl analgesia in the 52.5˚C hot plate assay (0-0.4 mg/kg, i.p.), implicating a neural mechanism selective for dopaminergic reward circuitry. To gain further insight into the neural mechanism, we are testing the hypothesis that *Csnk1e* knockout mice show differential DARPP-32 signaling in response to fentanyl. Finally, we are using an unbiased transcriptome approach (mRNA sequencing) to generate novel hypotheses regarding the molecular mechanism that mediates Csnk1e-mediated inhibition of opioid reward.

EVIDENCE FOR A TRKB-ACTIVATED P75NTR, JAK2, AND STAT3 COMPLEX IN NEURONS

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The etiology of intractable epilepsy is poorly understood. Studies show altered expression of gamma-aminobutyric acid receptor (GABAR) subunits may influence seizure susceptibility. Increased levels of brain derived neurotrophic factor (BDNF) are linked to seizure activity. Our laboratory previously found that BDNF upregulates α4 subunits and downregulates α1, producing a receptor isoform that desensitizes rapidly upon activation. Downregulation of α1 results from phosphorylation of janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3), followed by downstream interactions between inducible cAMP early repressor (ICER) and phosphorylated cAMP response element (pCREB). We wished to understand how JAK/STATs interact with BDNF signaling through tropomyosin receptor kinase B (TrkB) and neurotrophin receptor P75 (P75NTR). We used *in vitro* protein analysis of recombinant JAK and P75NTR in cell lines and primary neuronal cultures. Through TrkB inhibition, we found that activation of JAK/STAT signaling pathway requires BDNF-mediated activation of TrkB and the presence of P75NTR. Nerve growth factor (NGF) treatment did not activate JAK/STAT signaling, suggesting TrkB specificity. Co-immunoprecipitation shows endogenous TrkB coupled to P75NTR, P75NTR coupled to JAK2, and P75NTR coupled to STAT3. We confirmed this in HEK over-expressing recombinant P75NTR-Flag and JAK2-GFP. In immunocytochemical assays with JAK2-GFP over-expression, endogenous P75NTR co-localized more with JAK2 after BDNF treatment. These results suggest an interaction between P75NTR and JAK2, which may increase upon BDNF-activation of TrkB, and potentially be mediated by STAT3. This novel relationship between TrkB, JAK/STAT, and P75NTR may be important in epileptogenesis and JAK/STAT could be targeted for seizure therapeutics.

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UBIQUITINATION-MEDIATED DENDRITIC PRUNING BY THE ANGELMAN SYNDROME PROTEIN E6AP

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Department of Biology

Following growth and extensive branching of dendrites, structural maturation of neurons is characterized by a pruning process in which the extra, non-functional dendritic branches are actively removed. The timing of pruning and selection of specific dendrites determines the wiring of neural circuits and, ultimately, brain function. Angelman Syndrome (AS) and some autism spectrum disorders (ASD) are caused by genetic disruption of E6AP, an E3 ligase that targets multiple proteins for ubiquitination and proteasome-mediated degradation. However, the neuronal function of E6AP remains largely unknown. Using cultured rat hippocampal neurons, we find that expression of E6AP leads to a reduction in dendritic branches via dendritic pruning. We show that E6AP causes activation of the caspase proteases, a pathway known for local neurite degeneration and spine pruning. E6AP targets the endogenous inhibitor of apoptosis XIAP for ubiquitination and degradation, therefore decreasing the inhibition of caspases and inducing the pruning process. Meanwhile, E6AP expression also results in activation of the PI3K/Akt cascade, presumably functioning as a pro-survival measure antagonizing caspase-dependent local cell death. Given that dendritic pruning is an activity and experience-dependent developmental process, our findings well correlate with AS patient history which usually shows normal birth but delayed developmental milestones including intellectual and cognitive function, motor control, and speech. These findings provide novel mechanistic insight into our understanding of the physiological function of E6AP and the pathogenesis of Angelman syndrome and ASDs. Additionally, this study provides the first *in vitro* model of dendritic pruning in mammalian neurons.

FUNCTIONAL SIGNIFICANCE OF THE INTERACTION BETWEEN INDUCIBLE COSTIMULATOR (ICOS) AND ITS LIGAND (ICOSL)

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Inducible costimulator (ICOS) and its ligand (ICOSL) are a pair of costimulatory molecules that co-localize in germinal centers and are implicated in systemic lupus erythematosus (SLE). It is known that ICOSL sheds from the cell membrane and that the soluble form of ICOSL (sICOSL) is elevated in SLE; though the function of sICOSL is poorly understood. In addition to the known mechanism where ICOSL interaction with ICOS on T cells leads to cell signaling resulting in T cell activation and differentiation, there is also preliminary evidence that reverse signaling may also occur through ICOSL in antigen presenting cells. The ICOS: ICOSL binding interaction has not been fully characterized.

Experiments showed that sICOSL dimerized over time and with increasing temperatures and that the sICOSL: ICOS interaction did not follow a typical 1:1 binding interaction. In-solution binding experiments resulted in a tighter equilibrium dissociation binding constant (K_D) than the surface-based results. The K_D for ICOS binding to human ICOSL expressed on cells agreed well with the K_D for ICOS to the sICOSL, indicating that the in-solution binding measurement may measure binding avidity rather than affinity and that this may be the more physiologically relevant interaction. As this interaction is implicated in SLE, it would be useful to better understand the most relevant physiological form of these molecules (soluble or transmembrante) and the biological signaling events that are initiated via this interaction in order to determine whether targeting ICOS or ICOSL may be therapeutically viable approaches.

PREGNENOLONE SULFATE AS A MODULATOR OF SYNAPTIC PLASTICITY

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Pharmacology

Neurosteroids such as pregnenolone (PREG) have been implicated in the pathophysiology of neuropsychiatric disorders such as schizophrenia and cannabinoid addiction (Marx et al., 2009; Valée et al, 2014) as well as TBI, PTSD, anxiety disorders and epilepsy based on a number of small-scale clinical trials. Our laboratory discovered that PregS (pregnenolone sulfate) modulates excitatory synaptic transmission by modulating NMDAR (N-methyl-D-aspartate receptor) and Glur1 (AMPA receptor subunit) activity at micromolar concentrations using *in vivo* and *in vitro* preparations. Here we demonstrate a novel high affinity neuromodulatory effect of 50pM PregS on synaptic plasticity, within the physiological range (bulk concentrations of 26nM; Rustichelli et al., 2013). PregS induces an increase in intracellular Ca++ in cultured rat cortical neurons with an EC_{50} (the half maximal effective concentration) of ~2pM and an Emax (maximal concentration) of 50pM. PregS at 50pM increases colocalization of GluR1 (AMPA receptor subunit) and surface NR2A (NMDA receptor 2A subunit) colocalization with PSD95 (postsynaptic density protein 95) on dendritic spines within 10 minutes, suggesting increased surface-synaptic AMPARs and NMDARs. Whole cell recordings of primary hippocampal neurons revealed that PregS increases the frequency of sEPSCs (spontaneous excitatory postsynaptic currents), suggesting an increase in glutamate release from presynaptic terminals. MAP2 (Microtubule-associated protein 2) immunostaining and live imaging of eGFP (enhanced green fluorescent protein) transfected hippocampal neurons show that 50pM PregS 10min does not change dendritic spine density. The results demonstrate that physiologically relevant concentrations of PregS recruit AMPA and NMDA receptors at postsynaptic sites thereby activating neuronal excitability and synaptic plasticity.

AAV2/1-MEDIATED HIPPOCAMPAL GENE DELIVERY OF CD200 ENHANCES NEUROGENESIS AND AMYLOID CLEARANCE IN ALZHEIMER'S DISEASE MOUSE MODEL

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder in the world characterized by build-up of amyloid-β peptide (Aβ) and neurofibrillary tangle formation, inflammatory changes, and microglial activation. The exact mechanism of the elevated inflammatory changes are unknown. CD200 is a neuron-specific anti-inflammatory glycoprotein, and its receptor is expressed in glia. Both AD patients and mouse models show an age-related or Aβ-induced reduction in microglial CD200 expression, which may be the mechanism for elevated inflammation in the aged AD brain. The goal of this study was to determine if neuronal expression of CD200 enhances Aβ clearance and restores hippocampal neurogenesis in transgenic mice expressing Swedish APP mutant (Tg2576). Tetracycline-inducible adeno-associated virus serotype 2/1 expressing full-length CD200 (AAV-CD200) was bilaterally injected into the hippocampal region at the pre-symptomatic disease stage. Mice were then sacrificed at 12 months of age. Neurogenesis was measured in the brain using proliferation (BrdU) and differentiation markers (Dcx). Mice were assessed for Aβ loads by immunohistochemistry and ELISA. Neurogenesis was then examined in vitro by co-culture of neural stem cells with microglia stimulated by CD200 with or without Aβ. CD200 expression in transgenic mice enhanced proliferation and differentiation of neural stem cells and reduced Aβ load in the hippocampus. Nitric oxide synthase 2 was elevated in the hippocampi of AAV-CD200-injected transgenic mice, which may explain the enhanced phagocytosis of Aβ by microglia. These data indicate that CD200 may counteract age-dependent AD-like disease progression, thus giving it potential as a therapeutic against neurodegeneration.

IMPAIRED RENAL NCC FUNCTION AND EXPRESSION: A MECHANISM DRIVING NOREPINEPHRINE EVOKED SALT-SENSITIVE HYPERTENSION?

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Department of Pharmacology and Experimental Therapeutics

Aim: These studies test the hypothesis that impaired renal NCC regulation is a mechanism underlying the development of norepinephrine (NE) evoked salt-sensitive hypertension in Sprague-Dawley (SD) rats.

Methods: SD rats receiving a s.c. saline vehicle, NE (600ng/min), DMSO/saline vehicle (50:50), or hydrochlorothiazide (HCTZ 4mg/kg/d) and NE infusion were fed a 0.4% (NS) or high 8% NaCl (HS) diet for 14 days. On day 14, MAP, MAP response to i.v. hexamethonium (30mg/kg) and peak natriuresis to i.v. HCTZ (2mg/kg) infusion was assessed and NCC immunoblotting was performed on kidney cortex tissue.

Results: NE significantly increased MAP and vascular sympathetic tone. HS exacerbated NE induced hypertension, induced up-regulation of NCC protein levels and prevented dietary sodium stimulated reduction in HCTZ evoked natriuresis. HCTZ + NE co-infusion abolished the salt-sensitive component of NE induced hypertension.

	s.c. saline vehicle		s.c. NE		s.c. DMSO/ saline	s.c. NE + HCTZ
	NS	HS	NS	HS	HS	HS
MAP (mmHg)	124±2	124±1	151±3ϕ	171±4*≠	134±4	152±3≠ψτ
Peak ΔMAP to hexamethonium (mmHg)	-61±4	-66±3	-77±6ϕ	-81±8≠	-57±4.5	-69±3τ
Peak ΔUNaV to HCTZ (μeq/min)	8.7±0.3	7.2±0.7*	11.1±1.1 ϕ	10.8±0.4≠	6.3±1.3	0.4±0.2 ≠ψτ
NCC expression (ODU/mm² normalized to GAPDH)	TBD	TBD	0.15±0.02	0.27±0.06*	n.d.	n.d.

*p<0.05 vs respective NS gp; ϕp<0.05 vs vehicle NS gp; ≠p<0.05 vs vehicle HS gp; ψp<0.05 vs DSMO vehicle; τp<0.05 vs NE + HS gp; n=4-6/gp.

Conclusion: These findings reveal that the combination of high salt intake and excess NE evokes the development of salt-sensitive hypertension which consequently results in impaired NCC function and increased protein expression. These results contrast the established down-regulation of NCC expression and circulating NE levels observed during high salt-intake in salt-resistant rat phenotypes and provides a new insight into the mechanisms underlying the influence of sympathoexcitation on the kidney in hypertension.

miR-155 CRITICALLY REGULATES INFLAMMATION-INDUCED SUPPRESSION OF NEUROGENESIS IN THE DENTATE GYRUS

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Neurodevelopmental psychiatric disorders, including Down syndrome (DS) and autism spectrum disorders (ASD), are estimated to affect over 2 million people in the U.S. and thus pose an immense burden to society. Neuroinflammation and abnormalities in neurogenesis are key features of these disorders, and animal models have demonstrated that pro-inflammatory challenge suppresses neurogenesis. A growing body of evidence implicates microRNAs (miRNAs) in regulation of the peripheral acute inflammatory response. To elucidate the influence of miRNAs on the acute CNS inflammatory response, primary murine microglia were stimulated with lipopolysaccharide (LPS), a pro-inflammatory Toll-Like Receptor 4 ligand, and profiled for gene and miRNA expression. miR-155 and interleukin-6 (IL-6) were the most up-regulated miRNA and mRNA identified in LPS-stimulated microglia when compared to control. Using *in vitro* and *in vivo* methods, we found that miR-155 critically regulates IL-6 gene induction in microglia under inflammatory challenge. In *in vitro* co-culture studies, LPS-stimulated microglia co-cultured with neural stem cells led to skewing towards gliogenesis, whereas blockade of IL-6 or genetic ablation of miR-155 in microglia restored neural differentiation. Moreover, nestin promoter-driven Cre recombinase-mediated expression of miR-155 in neural and hematopoietic stem cells led to striking abnormalities in neurogenesis, morphology and location of neural stem cells in the hippocampal dentate gyrus. Our findings demonstrate that miR-155 regulates inflammation-induced neurogenic deficits, and reveal the miR-155/IL-6 axis as a highly promising therapeutic target for neurodevelopmental disorders.

TRANSCRIPTOME ANALYSIS AND GENE TARGETING OF A QTL INFLUENCING METHAMPHETAMINE SENSITIVITY

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Support: R00DA029635, R01HG005692, T32GM00A541, 9550300872 (Burroughs Wellcome Fund)

We previously utilized interval-specific congenic lines derived from C57BL/6J (B6) and DBA/2J (D2) alleles to fine map a quantitative trait locus (QTL) influencing methamphetamine (MA)-induced locomotor activity. We identified a 0.23 MB critical interval on chromosome 11 containing only two protein-coding genes, *Rufy1* and *Hnrnph1.* Notably, *Rufy1* contains three missense SNPs and Hnrnph1 contains 1 SNP near the 5' UTR. We are currently generating null mutant lines for both genes using transcription activator-like nucleases (TALENs) to determine the quantitative trait gene(s) that influence MA sensitivity. In an effort to identify the molecular mechanisms that bridge genetic variation with behavior, we conducted transcriptome analysis via mRNA sequencing (RNA-seq) in a B6.D2 congenic line (chr.11: 50-60 Mb) that captures the QTL. There was an overrepresentation of *cis*-regulated, differentially expressed genes within the congenic interval (4 out of 92 differentially expressed genes; FDR < 0.05) and widespread genomic regulation on all autosomes. Using Ingenuity Pathway Analysis (IPA), the top canonical pathways were "glutamate receptor signaling" and "GalphaQ signaling," while our top gene networks were "Behavior, Nervous System Development and Function, Tissue Morphology" and "Behavior, Neurological Disease, Cell-to-Cell Signaling and Interaction." In both networks, brain-derived neurotrophic factor (*Bdnf*) was a central, down-regulated gene ($p = 2.3 \times 10\text{-}5$). Future studies will be designed to test the hypothesis that the decreased MA-induced locomotor activity is caused by several convergent mechanisms, including both a deficit in the development and sustenance of dopaminergic neurons as well as a decrease in glutamate and adrenergic transmission and signaling in response to MA.

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THE EFFECT OF MYOSIN REGULATORY LIGHT CHAIN PHOSPHORYLATION ON β-MYOSIN MECHANICS IN HEALTH AND DISEASE

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Familial Hypertrophic Cardiomyopathy (FHC) is characterized by left ventricular hypertrophy and myofibrillar disarray. The clinical presentation of FHC varies from asymptomatic to progressive heart failure to sudden cardiac death. FHC is caused by mutations in genes that encode for sarcomeric proteins. There are 12 known FHC-linked mutations in the myosin regulatory light chain (RLC). The RLC mechanically stabilizes the myosin lever arm, which is crucial to myosin's ability to transmit contractile force. Two FHC RLC mutations, N47K and R58Q, have previously been shown to reduce myosin force production, stemming from a more compliant lever arm (Greenberg 2010). In contrast, phosphorylation of the RLC can impart stiffness to the myosin lever arm. We hypothesized that phosphorylation of the FHC-RLC may mitigate distinct mutation-induced structural and functional abnormalities. To generate mutant β-myosin, native RLC was depleted from porcine cardiac myosin and reconstituted with mutant or wild-type human RLC. In the work presented here, *in vitro* motility assays were utilized to investigate the effects of RLC phosphorylation on the FHC-RLC phenotype in the presence of an α-actinin frictional load. Myosin bearing FHC-RLCs reduced actin sliding velocity compared to WT when incubated with α-actinin, resulting in a ~30% reduction in force production. RLC Phosphorylation increased sliding velocity for all myosins, and restored mutant myosin force production to WT values. These results point to RLC phosphorylation as a general mechanism to increase force production of the individual β-myosin motor and as a potential target to ameliorate the FHC-RLC phenotype at the molecular level.

UNDERSTANDING REGULATION OF EIF2B BY EIF2

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Eukaryotic translation initiation factor 2B (eIF2B), the guanine nucleotide exchange factor for the G-protein eIF2, is one of the main targets for regulation of protein synthesis. eIF2B activity is inhibited in response to a wide range of stress factors and diseases, including viral infections, hypoxia, nutrient starvation, and heme deficiency, collectively known as the integrated stress response (ISR). eIF2B has five subunits: α through ε. The α-, β-, and δ-subunits are homologous to each other and form the eIF2B regulatory subcomplex, which is believed to be a trimer consisting of monomeric α-, β-, and δ-subunits. Phosphorylation of the α-subunit of the G-protein eIF2 in response to cellular stresses results in a tighter, non-productive binding to eIF2B. Here we present evidence that the regulatory subcomplex of eIF2B is in fact a hexamer consisting of an α homodimer and two βδ heterodimers. We also present evidence that phosphorylation of eIF2α induces a rearrangement of its N- and C-terminal domains, resulting in a modification of the eIF2B binding interface. This rearrangement is important for the observed conversion of eIF2(α-P) into a competitive inhibitor for eIF2B. Understanding the architecture of eIF2B and its interactions with eIF2 and eIF2(α-P) would not only elucidate the basis for a number of mutations known to result in pathological states, but could also open up this key regulatory process to therapeutic intervention.

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AMYLOIDOGENIC MUTATIONS IN HUMAN APOLIPOPROTEIN A-I ARE NOT ALWAYS DESTABILIZING: NEW INSIGHTS INTO APOA-I MISFOLDING IN FAMILIAL AMYLOIDOSIS AND ATHEROSCLEROSIS

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High-density lipoproteins (HDLs) and their major protein apolipoprotein A-I (apoA-I) remove excess cell cholesterol and protect against atherosclerosis. However, in acquired amyloidosis that is common in aging, non-variant full-length apoA-I deposits as fibrils in arteries contributing to atherosclerosis. In familial amyloidosis (AApoAI) N-terminal fragments of variant apoA-I deposit as fibrils in vital organs causing damage. There is no cure for apoA-I amyloidosis and its structural basis is unknown. Previously, we mapped AApoAI mutations on the crystal structure of C-terminally truncated human apoA-I to propose the first molecular mechanism of apoA-I misfolding in which protein destabilization and increased solvent exposure of the extended strand 44-55 initiate beta-aggregation. Here we test this hypothesis by analyzing two amyloidogenic mutations, G26R and W50R, inside or near this segment. Our surprising finding that AApoAI mutations are not necessarily destabilizing prompted us to search for additional triggers of apoA-I misfolding. Mapping residue segments predicted to promote or prevent beta-aggregation on the crystal structure suggests that the primary step in familial and acquired apoA-I amyloidosis is misfolding and aggregation of full-length lipid-free protein, triggered by perturbed local packing in the amyloid-prone segments; this can be followed by proteolysis. Multiple lines of experimental evidence support this idea that shifts the paradigm of AApoAI, as the disease is thought to result from release of the N-terminal proteolytic fragments followed by their misfolding. In sum, our biophysical studies suggest the first common molecular mechanism of apoA-I misfolding in various types of amyloidosis.

STRUCTURAL STUDIES OF ATP BINDING CASSETTE TRANSPORTER A1 AND ITS LIPID ACCEPTOR APOLIPOPROTEIN A-I

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Plasma high-density lipoprotein (HDL) levels are inversely related to the risk of cardiovascular disease. ATP-binding cassette transporter A1 (ABCA1) plays an important role in HDL formation. In peripheral cells, ABCA1 mediates the efflux of cellular cholesterol and phospholipids to plasma lipid-poor apolipoproteinA-I (apoA-I). Mutations in the ABCA1 and apoA-I genes can lead to severe HDL deficiency which may exacerbate cholesterol deposition in peripheral tissues and increase susceptibility for atherosclerosis. Thus, it is of vital importance to understand how ABCA1 and apoA-I functions in the initial step of HDL formation. However, despite intensive studies over years, the mechanism of the function of ABCA1 still remains unclear. The objective of the proposed study is to elucidate this mechanism from a structural perspective. We aim to achieve this goal by pursuing the following two specific aims: 1. to characterize the interactions between ABCA1 and its lipid acceptor apoA-I; 2. to determine the molecular structure of ABCA1. These studies will provide us detailed structural and functional information on ABCA1, thereby significantly enhancing our understanding of the process of HDL formation. Furthermore, this knowledge may facilitate the development of molecularly based strategies to control cardiovascular disease related to dysfunctions of lipid metabolism.

A PRESSURE-DEPENDENT MODEL FOR THE REGULATION OF LIPOPROTEIN LIPASE BY APOLIPOPROTEIN C-II

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Apolipoprotein C-II (apoC-II) is the co-factor for lipoprotein lipase (LPL) at the surface of triacylglycerol-rich lipoproteins. LPL hydrolyzes triacylglycerol, which increases local surface pressure as fatty acid accumulates at the surface. To understand how apoC-II adapts to these pressure changes, we characterized the behavior of apoC-II at multiple lipid/water interfaces. ApoC-II adsorption to a triacylglycerol/water interface resulted in large increases in surface pressure to equilibrium values. Interfacial compressions increase surface pressure and mimic the actions of LPL. On compressions, apoC-II rapidly desorbed at a retention pressure slightly higher than equilibrium. When the pH of the system approached the protein's pI, apoC-II desorption occurred at a higher surface area, but similar retention pressure. This result suggests that a greater amount of peptide binds the interface, but the retention pressure of apoC-II is independent of its surface concentration. We characterized apoC-II at phospholipid/triacylglycerol/water interfaces, which more closely mimic lipoprotein surfaces. ApoC-II had a large exclusion pressure at these interfaces. This indicates that apoC-II is able to bind phospholipid/triacylglycerol/water interfaces of high initial pressures. ApoC-II desorbed on interfacial compressions and re-adsorbed following re-expansions. However, apoC-II re-adsorption increased pressure by lower amounts than its initial adsorption. This suggests that apoC-II removes phospholipid on desorption. Altogether, our results suggest that apoC-II could regulate LPL activity in a pressure-dependent manner. ApoC-II binds triacylglycerol-rich lipoproteins of various initial pressures and serves as a co-factor for LPL as local pressure increases. At its retention pressure, apoC-II desorbs rapidly, removing phospholipid and slowing the activity of lipoprotein lipase.

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EXPRESSION AND PURIFICATION OF A TYPE 4B SECRETION SYSTEM CHAPERONE COMPLEX, icmSW, FROM LEGIONELLA PNEUMOPHILA

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Legionella pneumophila, a gram negative facultative intracellular bacteria, is the causative agent of Legionaires' disease. In order to survive in human alveolar cells the bacterium assembles a Type IVb secretion system that is used to deliver over 250 effectors into the host cell. These effectors modulate the host cell's processes allowing the pathogen to evade normal cellular defense systems. Two proteins, icmS and icmW, form a chaperone complex that deliver effectors to the protein translocase. Once at the membrane, the chaperone interacts with the core complex as well as a coupling complex. The purpose of this study is to find conditions for expression and purification of a stable, soluble complex in order to structurally characterize this chaperone. Two methods have been used to isolate these proteins: co-refolding from inclusion bodies and expression as a soluble MBP fusion partner. Additionally, based on information from literature and collaborations, we have identified a peptide and truncated protein that bind to icmS and icmW and may stabilize the complex, making it suitable to crystallography. The peptide (WipA-378-420) and truncated protein (DotL-631-783) are derived from an effector and a Type IVb coupling protein respectively. Both the peptide and truncated protein have been tested with different fusion partners to access their expression and solubility.

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EXAMINATION OF POLIOVIRUS RNA REPLICATION COMPLEX REVEALS UNIQUE MEMBRANE MORPHOLOGIES

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Replication of positive-sense RNA viruses depends on alterations to host cell membranes. A complex termed a replication organelle is assembled from host cell membranes and proteins in complex with viral proteins to form a platform for replication. Poliovirus is a prototypical positive-sense RNA virus, and the core of the replication organelle can be isolated from the lysates of infected cells. In this work we investigate the ultrastructural details of the poliovirus replication complex using electron cryotomography in an attempt to understand the structural roles that membranes play in virus replication. Our data show complex, tightly associated membranous structures consisting of single and double membrane bilayers. Electron dense particles extending from membranes provide contacts between vesicles, and are expected to include both viral and cellular proteins. Invaginated membranous structures are produced by membrane-associated proteins and are proposed to provide protected surfaces for RNA replication.

DIRECT VISUALIZATION OF TROPOMYOSIN BINDING AND DETACHMENT FROM F-ACTIN

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Physiology & Biophysics

Tropomyosin (Tm) is an alpha-helical coiled-coil protein that binds along the length of F-actin in striated muscle thin filaments. Tropomyosin monomers polymerize head-to-tail, each one binding seven actin monomers, and studies have shown that tropomyosin completely saturates thin filaments. Together with the thin filament-associated protein, troponin, tropomyosin regulates the binding of myosin to actin in the thin filament via calcium binding to troponin. Tropomyosin cooperatively binds F-actin, however, the mechanism of how the low affinity tropomyosin monomer binds to and assembles on actin filaments to form a strongly bound tropomyosin chain remains unknown.

In this work, we used TIRF microscopy to monitor fluorescently-labeled skeletal and smooth muscle tropomyosin binding to actin. We found that random, weak monomer binding leads to the formation of "nucleation sites" with enhanced affinity for the actin filament—a process most likely dependent on tropomyosin end-to-end interactions. Stepwise changes in fluorescence intensity indicate that three tropomyosin molecules are required to form a stable nucleation site. From these sites, subsequent chain elongation is rapid and correlates with the strength of end-to-end tropomyosin linkages. Furthermore, upon rapid dilution of labeled tropomyosin, we observed tropomyosin detachment from "gaps" on actin filaments, where there were fewer than the requisite seven actin monomers required for Tm binding, as expected from the nucleation-polymerization binding mechanism. These studies provide insight into the processes of thin filament assembly and regulation, as well as how non-muscle tropomyosin isoforms may act to regulate the cell's cytoskeleton.

Notes