The 28th Annual

Henry I Russek Student Achievement Day

May 6, 2022

Hiebert Lounge

9:00am - 5:00pm

Keynote Address:

"The Human Microbiome in Health and Disease"

Eric Alm, PhD

Professor of Biological Engineering,
MIT and Broad Institute
Co-Director, Center for Microbiome Informatics and Therapeutics
Co-Director, Global Microbiome Conservancy

This day is in memory of Dr. Henry I. Russek, physician scientist, and Mrs. Elayne Russek, philanthropist, who recognized the importance of celebrating the accomplishments of young scientists as they become the next generation to cure disease and enrich society by their own example.

"Genius is perseverance in disguise."

BOSTON UNIVERSITY

BU School of Medicine
Graduate Medical Sciences

Welcome to the Twenty-Eighth Annual Henry I. Russek Student Achievement Day!

It is hard to believe that this is our 28th year. I am so happy we are back live to share our wonderful students together as we peek out from the darkness of one of the worst times in our history. Our students have been a beacon for us displaying such resilience and determination that this alone is cause for celebration. It also signals that indeed there is hope in the world because they see how life should be and they engage in activities that even at a local level have a ripple effect to increase compassion and a sense of duty to leave the world a better place.

This is their event so let's make it shine and stop the world if only for one day! I want to take the time to thank all the faculty and staff in all the nooks and crannies that have made Achievement Day possible for so many years. You all know who you are and please know you are so appreciated. This year I am dedicating the day to my mother Elayne Russek who stood on the podium with me for so many years and was so proud of being a part of Boston University's legacy. She is here in spirit today and will always be every year to rally our students in their exciting achievement journey.

Pura Vida,

Shelley J. Russek

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

Eric Alm, PhD

Professor of Biological Engineering, MIT and Broad Institute

Research-at-a-glance:

- Engineering microbial ecology to improve human health
- Engineering the human microbiome
- Non-invasive monitoring of human health
- Environmental surveillance
- Sewage epidemiology

Biography

Eric Alm earned his Bachelors from the University of Illinois (Champaign-Urbana), his Masters from the University of California (Riverside), and his PhD from the University of Washington (Seattle). He held a postdoctoral appointment at the University of California (Berkeley) and Lawrence Berkeley National Lab before joining the faculty at MIT. His research group is an interdisciplinary team of computer scientists, computational biologists, molecular biologists, and microbial ecologists.

Research

The human microbiome plays a key role in human health and disease. Research in my group includes both computational/theoretical and experimental approaches to understanding and engineering the human microbiome. Our research is focused on translating basic science discoveries rapidly into the clinic, where they can contribute to better outcomes for patients. Some areas of special interest include:

- Developing therapeutics based on synthetic microbial communities
- Personalized medicine
- Monitoring human activities through Smart Sewers
- Smart Toilets that track human health
- Discovering low-cost non-invasive biomarker

Research Areas

- Biophysics
- Computational Modeling
- Energy
- Macromolecular Biochemistry
- Microbial Systems
- Omics
- Synthetic Biology
- Systems Biology

Student Achievement Day 2022

Program of Events

Coffee and pastries available at 8:30 a.m. Please pick up badges in Heibert lounge from student hosts.

9:15-9:30 a.m.

Welcoming addresses by Dr. C. James McKnight, Associate Provost & Dean of Graduate Medical Sciences and Dr. Shelley Russek, President of the Russek Foundation, Department of Pharmacology and Experimental Therapeutics

9:30-10:30 a.m.

Henry I. Russek Keynote Lecture by Dr. Eric Alm, Professor of Biological Engineering, Massachusetts Institute of Technology and the Broad Institute, "*The Human Microbiome in Health and Disease*"

10:30 a.m.-1:30 p.m.

Poster Session: Presented by graduate students enrolled in Graduate Medical Sciences (Bag luncheon available at 11:30 a.m.). (Winners will have award stickers on their posters.)

11:30 a.m.-12:30 p.m.

Award winners (First, Second, Third place and Moderators) please get your lunch and come to the stage in the Hiebert Lounge. You will be having lunch with our Keynote Speaker & Visiting Professor Dr. Eric Alm.

1:30-3:10 p.m.

Oral Session: Slide presentations by the Henry I. Russek Student Achievement First Prize Recipients. (Each presentation is 8 min. with an additional 2 min. for questions.)

3:10 p.m.

Award presentations by Dr. Shelley J. Russek, Russek Foundation, and photos of our award winners!

Student Presentations (1:30 p.m. - 3:10 p.m.)

1:30-1:40 p.m.
Katharine Babcock: INTERFACE ASTROGLIOSIS IN CONTACT SPORT HEAD IMPACTS AND MILITARY BLAST EXPOSURE (Anatomy & Neurobiology, Advisor: A. McKee)

1:40-1:50 p.m.
Megan Snyder: MALIGNANT CELL EXPRESSION OF THE ARYL HYDROCARBON RECEPTOR INDUCES PD-L1 AND IMMUNOSUPPRESSION IN MODELS OF ORAL AND LUNG CANCER (Program in Genetics & Genomics, Advisor: D. Sherr)

1:50-2:00 p.m.
Jacob Beierle: A REDUCED COMPLEXITY CROSS BETWEEN BALB/c SUBSTRAINS IDENTIFIES Zhx2 AS A CANDIDATE GENE UNDERLYING OXYCODONE METABOLITE BRAIN CONCENTRATION AND STATE-DEPENDENT LEARNING OF OPIOID REWARD (Pharmacology & Experimental Therapeutics, Advisor: C. Bryant)

2:00-2:10 p.m.
Joseph Kern: HIPPO PATHWAY INACTIVATION DRIVES BASAL-LIKE BREAST CANCER DEVELOPMENT (Biochemistry, Advisor: X. Varelas)

2:10-2:20 p.m.
Emily Lewkowicz: STRUCTURAL BASIS FOR THE ACTION OF HEPARAN SULFATE AND OTHER PERIODIC POLYANIONS AS UBIQUITOUS AMYLOID AGONISTS (Physiology & Biophysics, Advisor: O. Gursky)

2:20-2:30 p.m.
Anna Marin: THE USE OF EVENT-RELATED POTENTIALS AS A MARKER OF AMYLOID PET POSITIVITY AMONG PATIENTS FROM A MEMORY DISORDERS CLINIC (Behavioral Neuroscience Program, Advisors: A. Budson & K. Turk)

2:30-2:40 p.m.
Thomas Morin: FUNCTIONAL RECONFIGURATION OF A TASK-ACTIVE FRONTOPARIETAL CONTROL NETWORK FACILITATES ABSTRACT (Graduate Program for Neuroscience, Advisor: C. Stern)

2:40-2:50 p.m.
Yuhei Uda: PROTECTIVE ROLE OF PARATHYROID HORMONE SIGNALING IN OSTEOCYTES AGAINST OXIDATIVE STRESS (Orofacial & Skeletal Biology Program, Advisors: P. Divieti Pajevic)

2:50-3:00 p.m.
Anthony Yeung: DE-NOVO HEMATOPOIESIS FROM THE FETAL LUNG (Graduate Program in Molecular & Translational Medicine, Advisor: G. J. Murphy)

3:00-3:10 p.m.
Mengji Yuan: SATURATED FAT INTAKE FROM DAIRY SOURCES IS ASSOCIATED WITH REDUCED CARDIOVASCULAR RISK IN FRAMINGHAM OFFSPRING STUDY WOMEN (Nutrition & Metabolism Program, Advisors: L. L. Moore)

Recipients of the Henry I. Russek Student Achievement Awards 2022

First Place

Katharine Babcock
Department of Anatomy & Neurobiology
Advisor: A. McKee

Anna Marin
Behavioral Neuroscience Program
A. Budson & K. Turk

Joseph Kern
Department of Biochemistry
Advisor: X. Varelas

Megan Snyder
Program in Genetics & Genomics
Advisor: D. Sherr

Thomas Morin
Graduate Program for Neuroscience
Advisors: C. Stern

Anthony Yeung
Graduate Program in Molecular & Translational Medicine
Advisor: G. J. Murphy

Mengji Yuan
Nutrition & Metabolism Program
Advisors: L. L. Moore

Yuhei Uda
Orofacial and Skeletal Biology Program
Advisors: P. Divieti Pajevic

Jacob Beierle
Department of Pharmacology & Experimental Therapeutics
Advisor: C. Bryant

Emily Lewkowicz
Department of Physiology & Biophysics
Advisor: O. Gursky

Second Prize

Alexandra Tsolias
Department of Anatomy & Neurobiology
Advisor: M. Medalla

Adeline Matschulat
Department of Biochemistry
Advisor: X. Varelas

Jiaji Chen
Program in Genetics & Genomics
Advisor: R. Dries

Shen Ning
Graduate Program for Neuroscience
Advisors: A. Ho, R. Tanzi, & D.-Y. Kim

Elissa Everton
Program in Molecular & Translational Medicine
Advisor: G. J. Murphy

Chelsea Webber
Department of Pharmacology & Experimental Therapeutics
Advisor: B. Wolozin

Andrew Chang
Department of Physiology & Biophysics
Advisor: C. Gabel

Third Prize

Renee Groechel
Department of Anatomy & Neurobiology
Advisor: R. Killiany

Jarrod Moore
Department of Biochemistry
Advisor: A. Emili

Lucas Carstensen
Graduate Program for Neuroscience
Advisors: M. Hasselmo & D. Phil

Elim Na
Program in Molecular & Translational Medicine
Advisor: L. Quinton

Xuan (Anita) He
Department of Pharmacology & Experimental Therapeutics
Advisor: S. Fisher

Table of Participating Abstracts

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

Katharine Babcock (*)
Karen Bottenfield (22)
JoColl Burgess (18)
Morgane Butler (29)
Natalie Campbell (30)
Sarah DeVries (32)
Renee Groechel (31***)
Songjun (William) Li (35)
Alexandra Tsolias (23)

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INTERFACE ASTROGLIOSIS IN CONTACT SPORT HEAD IMPACTS AND MILITARY BLAST EXPOSURE

K. Babcock, B. Abdolmohammadi, P. Kiernan, I. Mahar, J. Cherry, V. Alvarez, L. Goldstein, T. Stein, A. McKee, and B. Huber

Department of Anatomy and Neurobiology

Exposure to military blast and repetitive head impacts (RHI) in contact sports is associated with increased risk of long-term neurobehavioral sequelae and cognitive deficits, and the neurodegenerative disease chronic traumatic encephalopathy (CTE). At present, the exact pathogenic mechanisms of RHI and CTE are unknown, and no targeted therapies are available. Astrocytes have recently emerged as key mediators of the multicellular response to head trauma. Here, we investigated interface astrogliosis in blast and impact neurotrauma, specifically in the context of RHI and early stage CTE. We compared postmortem brain tissue from former military veterans with a history of blast exposure with and without a neuropathological diagnosis of CTE, former American football players with a history of RHI with and without a neuropathological diagnosis of CTE, and control donors without a history of blast, RHI exposure or CTE diagnosis. Using quantitative immunofluorescence, we found that astrogliosis was higher at the grey-white matter interface in the dorsolateral frontal cortex, with mixed effects at the subpial surface and underlying cortex, in both blast and RHI donors with and without CTE, compared to controls. These results indicate that certain astrocytic alterations are associated with both impact and blast neurotrauma, and that different astroglial responses take place in distinct brain regions.

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PROPARGYLCHOLINE TO ASSESS NEWLY SYNTHESIZED MYELIN IN THE RHESUS MONKEY BRAIN AFTER CORTICAL INJURY

K. Bottenfield, T. L. Moore, F. Mortazavi, and D. L. Rosene

Department of Anatomy and Neurobiology

Myelin constitutes approximately 40 percent of the primate forebrain and damage impairs neural function. Myelin can be assessed by electron microscopy, histology, immunohistochemistry (MBP), and a label free confocal method (SCoRe). Each has advantages but none offer a reliable, validated method to assess myelin maintenance or repair. Newly synthesized myelin can be labeled by *in vivo* administration of propargylcholine (P-Cho). It is incorporated into phosphatidylcholine and sphingomyelin, major components of myelin. P-Cho contains an alkyne moiety that can be conjugated with a fluoro-labeled azide and visualized with confocal microscopy. Here we report, for the first time, the use of P-Cho to assess myelin synthesis following cortical injury. P-Cho was administered after injury by IP injections (3 – 4.25 mg/kg) daily for 6 days in 6 young adult monkeys at 1, 2, and 6 weeks before euthanasia. Fixed frozen brain sections were processed using Click-iT technology to conjugate P-Cho with a fluorescent azide. To quantify new myelin this was combined with immunohistochemical labeling of myelin with antibody to MBP. Results show stable P-Cho labeling from 1 to 6 weeks. In quantifying new myelin after injury to the primary motor cortex, results show significantly greater P-Cho and its co-localization with MBP in the underlying white matter of the ipsilesional hemisphere when compared with the contralateral hemisphere. This validates P-Cho for assessing myelin plasticity in the nonhuman primate brain and how it might be used to assess pharmacotherapies aimed at inducing remyelination and enhancing myelin synthesis. (Supported by NIH grants R21-NS111174, U01-NS076474 and RF1-AG043640).

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LONGITUDINAL RECORDINGS OF IN VIVO CALCIUM DYNAMICS OF BASOLATERAL AMYGDALA NEURONS DURING UNPREDICTABLE AND PREDICTABLE FEAR CONDITIONING

J. Burgess, L. F. Yee, S. Lim Hong, S. Shah, A. E. Rudolph, and K. Goosens

Department of Anatomy and Neurobiology

Pavlovian fear conditioning is used to explore the mechanisms that underlie aversive learning and memory, but it typically uses predictable sensory cues to induce learning. In the real world, cues that predict danger rarely have temporal predictability, and environmental unpredictability is known to enhance aversive memory. Regardless, it is not known how unpredictability leads to stronger aversive memories. The basolateral amygdala (BLA) is one brain region that contributes to aversive memory formation. We compare network dynamics in the BLA during predictable versus unpredictable fear conditioning. We injected AAV in the BLA of CB57BL/6 male mice to express GCAMP6s. A microendoscopic fiber and lens were placed over the BLA to visualize calcium activity during fear conditioning (Day 1) and a long-term auditory memory test (Day 4). Mice received either predictable or unpredictable fear conditioning. We found a sub-population of BLA neurons that exhibited elevated CS-

US calcium responses in fear conditioning and recall ("**Winners**"), which were also spatially clustered. Additionally, another neuronal population exhibited tone-shock convergence during training but failed to display tone responses during recall ("**Losers**"). The level of tone-shock convergence within single neurons was low across both Winners and Losers. Predictable and unpredictable training resulted in similar proportions of Winners and Losers, but Winners from mice that received unpredictable training exhibited more correlated activity during the tone, as well as greater 'emergent' convergence across Winner neighbor pairs. The number of neurons recruited to an aversive memory is stable across different memory strengths, but the local network dynamics supporting those memories differs in important ways.

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TAU PATHOLOGY IN CHRONIC TRAUMATIC ENCEPHALOPATHY IS PRIMARILY NEURONAL

M. Butler, V. Alvarez, M. Buckland, J. Cherry, E. Dixon, B. Huber, A. McKee, and T. Stein

Department of Anatomy and Neurobiology

Millions of individuals are exposed to repetitive head impacts (RHI) each year through contact sports, military blast, and interpersonal violence. RHI is the biggest risk factor for developing chronic traumatic encephalopathy (CTE), a debilitating neurodegenerative tauopathy with cognitive, mood, behavioral, and motor symptoms. Recent consensus criteria defined the pathognomonic CTE lesion as perivascular hyperphosphorylated tau (p-tau) neuronal aggregates with astroglial p-tau as a supporting but non-diagnostic feature of the disease. However, no comprehensive quantitative characterization of neuronal and astroglial p-tau pathology has been conducted in CTE. In the present study, spatial and cellular distribution of p-tau pathology was quantitated in post-mortem dorsolateral frontal cortex of 150 individuals with CTE, from ages 21 and 80 years, without comorbid pathology. P-tau immunoreactive cells were counted in the gray matter sulcus, crest, subpial region, and within pathognomonic lesions. Overall, significantly more neuronal p-tau than astrocytic p-tau was found across cortical regions ($p<0.0001$), and both neuronal and astrocytic p-tau were significantly elevated in the gray matter sulcus compared to the crest ($p<0.001$). Sulcal astrocytic p-tau was mostly (75%, $p<0.0001$) localized to the subpial region as age-related tau astrogliopathy (ARTAG). Scattered astrocytic p-tau was observed in the parenchyma and within pathognomonic lesions, but only in older individuals with severe disease. Analyses showed that astrocytic p-tau was significantly associated with age, but not years of exposure to RHI or CTE stage. These findings support neuronal degeneration as the driving feature of CTE and will help inform future research in the detection of neuronal degeneration in CTE.

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EFFECT OF EXOSOMES ON OLIGOCORTICAL SPHEROIDS IN DOWN SYNDROME

N. Campbell, C. Downs, M. Medalla, T. L. Moore, Y. Patel, and E. Zeldich

Department of Anatomy and Neurobiology

Extracellular vesicles (EVs) are released by most cell types and are important in inter-cellular communication. Abnormal EV signaling occurs in many conditions including Alzheimer's Disease (AD) and Down Syndrome (DS). EVs from stem cells have emerged as a possible intervention for different neurological conditions. Mesenchymal stromal cell-derived (MSCs) EVs from bone marrow of young healthy monkeys have been shown to reduce AD pathology in mouse models. Individuals with DS are disproportionately affected by early onset AD and investigating MSC-EVs to potentially mitigate AD pathology in DS is critical. This study explores EV applications on 3D human brain models of DS. We generated DS-derived oligocortical spheroids (OLS) containing neurons, astrocytes, and oligodendrocytes to investigate effects of EVs in physiologically relevant conditions. Trisomic OLS display higher levels of amyloid beta depositions, are smaller than their euploid counterparts and have elevated levels of cleaved-caspase 3 detection, indicating more cell death. EV-treated trisomic OLS demonstrated greater preserved cortical volume, decreased levels of amyloid beta, reduced cell death and higher counts of neurons expressing deep and superficial layer markers compared to untreated trisomic OLS. These results suggest that EVs alleviated AD-related pathology and promoted neurogenesis in DS-derived OLS. Our novel studies show the efficacy of MSC-EVs in mitigating DS and AD-related cellular phenotypes and pathological depositions in OLS. Furthermore, oligocortical spheroids present a unique tool for target validation of potential therapeutics.

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IMMUNE SIGNALS MODULATING MICROGLIAL PHAGOCYTOSIS MAY CONTRIBUTE TO SYNAPTIC LOSS AND RELATED COGNITIVE DEFICITS IN THE AGING MONKEY

S. A. DeVries, F. Mortazavi, M. Medalla, T. L. Moore, and D. L. Rosene

Department of Anatomy & Neurobiology

Normal aging in humans, even without neurodegeneration, is accompanied by varying degrees of cognitive decline. The rhesus monkey model of normal aging provides insight into underlying changes in the brain since monkeys display structural and cognitive changes similar to humans but are spared from neurodegeneration. While neurons are not lost with normal aging in humans or monkeys, we and others have shown myelin damage and synaptic loss in areas critical for cognition, such as the dorsolateral prefrontal cortex (dlPFC). Synaptic loss could be secondary to myelin damage, or result from aberrant synapse elimination by microglia, the resident immune cells of the CNS, that become dysregulated with age. Microglial phagocytosis is modulated by immune "eat me" and "don't eat me" signals that respectively initiate and inhibit phagocytosis. Previous research has focused on the "eat me" signals mediated by complement component C1q, leaving the "don't eat me" signals, such as CD47, largely unstudied. To investigate changes in signals initiating and inhibiting microglial phagocytosis, multilabel

immunofluorescence (IF) was used to examine the localization of CD47 and C1q on postsynaptic sites labeled by PSD95 in the dlPFC of 5 male and 6 female rhesus monkeys ranging from 8 to 28 years of age. All monkeys were tested on delayed non-match to sample (DNMS) and delayed recognition span task (DRST) that respectively measure recognition memory and working memory. Results revealed age-related decreases in CD47 IF mean intensity (p=0.042) and a concurrent increase in C1q intensity (p=0.036). IF showed decreased CD47-PSD95 colocalization (p=0.077) and increased C1q-PSD95 colocalization (p=0.038) in aged compared to young monkeys. Immuno-EM confirmed the localization of C1q on synaptic membranes. Interestingly, increased C1q-PSD95 colocalization was weakly associated with cognitive decline (p=0.10). These results suggest that with age, microglia receive decreased phagocytotic inhibition from CD47 along with increased phagocytotic signals from C1q, providing a possible mechanism for age-related synaptic loss and associated cognitive impairment.

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BIOMARKERS OF ALZHEIMER'S DISEASE IN BLACK PARTICIPANTS OF THE ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE

R. Groechel, Y. Tripodis, M. Alosco, J. Mez, W. Qui, A. Budson, L. Goldstein, N. Kowall, L. Shaw, M. Weiner, C. Jack Jr. and R. Killiany

Department of Anatomy & Neurobiology
For the Alzheimer's Disease Neuroimaging Initiative

Background and Objectives: Relatively little is known about dementia in the Black population. Our goal was to characterize clinical and biomarker profiles of Alzheimer's disease (AD) in Black or African American (BoAA) participants enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI).

Methods: Data for this cross-sectional study came from 85 BoAA ADNI participants. Data included participant demographics, 3T MRI scans, cognitive and functional performance, CSF profiles of amyloid-beta ($A\beta_{1-42}$), total tau (t-tau), phosphorylated tau (p-tau), and *APOE* ε4 status. Analyses of variance and covariance were used to assess the impact of clinical syndrome group (cognitively normal (CN), mildly cognitively impaired (MCI), or dementia) and the interaction between groups and *APOE* ε4 carrier status.

Results: Significant group differences were found in thickness of the entorhinal cortex ($F(3,81) = 14.06$, $p < 0.001$) and volume of the hippocampus ($F(3,81) = 13.38$, $p < 0.001$). Significant group differences were also found in 8 measures of cognition and function, CSF $A\beta_{1-42}$ ($F(2, 45) = 3.28$, $p = 0.047$), CSF t-tau ($F(2,45) = 7.25$, $p = 0.002$), and CSF p-tau ($F(2,45) = 7.4350$, $p = 0.002$). Interactions between clinical syndrome group and *APOE* ε4 carrier status were found on three measures.

Discussion: Our findings support the hypothesis that there would be an increase in AD biomarkers abnormalities between clinical syndrome groups. Post hoc testing revealed these differences were primarily between the CN and dementia groups but some differences were also apparent between the CN and MCI group and/or the MCI and dementia group.

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FRONTAL NEURONS DRIVING COMPETITIVE BEHAVIOR AND ECOLOGY OF GROUPS

S. Li, O. Zeliger, L. Strahs, R. Báez-Mendoza, M. Johnson, A. McDonald Wojciechowski, and Z. Williams

Department of Anatomy & Neurobiology

Determining how much effort to allocate when competing with others is essential to effective group living and hinges on the ability of individuals to integrate information not only about the resources available but also about the social rank and behavior of other group members to maximize benefit. Although frontal brain regions such as the anterior cingulate cortex (ACC) have been implicated in social competition, the single-cellular mechanisms that precisely drive competitive interactions or the behavior of social groups, however, remain poorly understood. Here we developed a naturalistic group paradigm in which large cohorts of mice competitively foraged for food as we wirelessly tracked neuronal activities across thousands of unique interactions. We found neurons in the ACC that adaptively represented the social rank of the animals in relation to others. Although social rank was closely behaviorally linked to success, these cells disambiguated the relative rank of the mice from their competitive behavior, and incorporated information about the resources available, the environment, and past success to influence their decisions. Using multiclass models, we show how these neurons tracked other group members and accurately predicted upcoming success. Using neuromodulation techniques, we also show how the neurons conditionally influenced competitive effort—increasing the effort of the animals only when they were more dominant to their groupmates and decreasing it when they were subordinate—effects that were not observed in other frontal lobe areas. Together, these findings reveal cingulate neurons that serve to utilize social information required to optimize success and adaptively drive competitive decision.

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MUSCARINIC ACETYLCHOLINE RECEPTOR LOCALIZATION ON DISTINCT EXCITATORY AND INHIBITORY NEURONS WITHIN THE ACC AND LPFC OF THE RHESUS MONKEY*

A. Tsolias[1] and M. Medalla[1, 2]

[1] Department of Anatomy & Neurobiology
[2] Center for Systems Neuroscience

Acetylcholine (ACh) can act on pre- and post-synaptic muscarinic receptors (mAChR) in the cortex to influence a myriad of cognitive processes. Two functionally-distinct regions of the prefrontal cortex–the lateral prefrontal (LPFC) and anterior cingulate (ACC)–are differentially innervated by ascending cholinergic pathways yet, the nature and organization of prefrontal-cholinergic circuitry in primates is not well understood. Using multi-channel immunohistochemical labeling and high-resolution microscopy, we found regional and laminar differences in the subcellular localization and the densities of excitatory and inhibitory subpopulations expressing $m1^+/m2^+$ muscarinic receptors in the supragranular layers of LPFC and ACC in rhesus monkeys. The subset of layer 3 (L3) $m1^+/m2^+$ expressing SMI-32^+ pyramidal neurons was denser in LPFC, while $m1^+/m2^+$ SMI-32^+ neurons co-expressing calbindin was greater in ACC.

Further, we found between-area differences in laminar $m1^+$ dendritic expression, and $m2^+$ presynaptic localization on cortico-cortical ($VGLUT1^+$) and sub-cortical inputs ($VGLUT2^+$), suggesting differential cholinergic modulation of top-down versus bottom-up inputs in the two areas. While almost all inhibitory interneurons–parvalbumin, calbindin and calretinin–expressed $m1^+$, the localization of $m2^+$ differed by subtype and area. The ACC had a greater proportion of $m2^+$ inhibitory neurons compared to the LPFC and a greater density of presynaptic $m2^+$ localized on inhibitory ($VGAT^+$) inputs targeting proximal somatodendritic compartments and axon initial segment of L3 pyramidal neurons. These data suggest a greater capacity for $m2^+$-mediated cholinergic suppression of inhibition in the ACC. The anatomical localization of mAChRs on ACC and LPFC micro-circuits contribute to our understanding of cholinergic neuromodulation in functionally-distinct prefrontal areas.

Behavioral Neuroscience Program

NOTE: Prize winners are noted in the following list of Abstracts by:

*1st Prize

The accompanying number indicates each abstract's poster board.

Participants
Anne Billot (4)
Jenna Groh (16)
Anna Marin (*)

4

PREDICTING POST-STROKE APHASIA RECOVERY FROM MULTIMODAL NEUROIMAGING AND BEHAVIORAL DATA

A. Billot, E. Braun, M. Betke, D. Caplan, J. Higgins, P. Ishwar, S. Kiran, A. Kurani, S. Lai, T. Parrish, B. Rapp, C. Thompson, and M. Varkanitsa

Behavioral Neuroscience Program

Background: Poststroke recovery depends on multiple factors and varies greatly across individuals. Using machine learning models, this study investigated the independent and complementary prognostic role of different patient-related factors in predicting response to language rehabilitation after a stroke.

Methods: Fifty-five individuals with chronic poststroke aphasia underwent a battery of standardized assessments and structural and functional magnetic resonance imaging scans and received 12 weeks of language treatment. Support vector machine and random forest models were constructed to predict responsiveness to treatment using pretreatment behavioral, demographic, and structural and functional neuroimaging data.

Results: The best prediction performance was achieved by a support vector machine model trained on aphasia severity, demographics, measures of anatomic integrity and resting-state functional connectivity (F1=0.94). This model resulted in a significantly superior prediction performance compared with support vector machine models trained on all feature sets (F1=0.82, $P<0.001$) or a single feature set (F1 range= 0.68–0.84, $P<0.001$). Across random forest models, training on resting-state functional magnetic resonance imaging connectivity data yielded the best F1 score (F1=0.87).

Conclusions: While behavioral, multimodal neuroimaging data and demographic information carry complementary information in predicting response to rehabilitation in chronic poststroke aphasia, functional connectivity of the brain at rest after stroke is a particularly important predictor of responsiveness to treatment, both alone and combined with other patient-related factors.

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DEFINING AND CHARACTERIZING GWI PATHOBIOLOGY USING LONGITUDINAL BRAIN IMAGING BIOMARKERS

J. Groh, C.-H. Cheng, B.-B. Koo, R. Killiany, M. Krengel, and K. Sullivan

Behavioral Neuroscience Program

Objective: Veterans of the 1991 Gulf War (GW) continue to experience chronic symptoms of Gulf War Illness (GWI) which includes fatigue, memory and concentration problems, muscle and joint pain and headaches. Brain white matter (WM) alterations have been shown to be present in veterans with GWI in several different studies suggesting a correlation to the disorder. The objective of this study is to assess if longitudinal brain volumetric changes are present in veterans with GWI. We hypothesized that veterans with GWI would have longitudinal patterns of decreased brain volumetrics and white matter structural integrity.

Participants and Methods: Study participants included 25 Gulf War veterans who met criteria for GWI based on the Kansas GWI definition. Each participant had MRI brain imaging performed at two time points on average five years apart on a Philips 3T scanner. The mean current age for participants was 56.5 years and included 28% women. For this study, longitudinal Time 1 and Time 2 MPRAGE MRI scans were compared. Cortical reconstruction and volumetric segmentation were performed with Freesurfer 6.0, which is documented and freely available for download. Paired t-tests were performed to evaluate changes in brain volumetrics over time within the same individuals.

Results: Veterans with GWI showed decreased hippocampal volume in the left ($p=0.001$) and right hemispheres ($p=0.001$) from Time 1 to Time 2. White matter pathways were also changed over time. In particular, the corpus callosum decreased across all segmented regions including the anterior region ($p=0.013$), mid-anterior region ($p=0.017$), central region ($p= 0.010$), mid-posterior region ($p= 0.002$), and the posterior region ($p=0.009$) from Time 1 to Time 2.

Conclusions: As hypothesized, individuals with GWI showed decreased volumetrics in key structures and white matter pathways over time. These white matter changes appear to be progressing as veterans' age. We have also noted cognitive changes in memory, attention and processing speed that may correlate with these brain volumetric changes. More research is needed in a larger study sample to confirm these preliminary longitudinal brain imaging results and to compare with cognitive outcomes.

*

THE USE OF EVENT-RELATED POTENTIALS AS A MARKER OF AMYLOID PET POSITIVITY AMONG PATIENTS FROM A MEMORY DISORDERS CLINIC

A. Marin, A. Budson, R. DeCaro, K. Schiloski, K. Turk, A. Vives Rodriguez

Behavioral Neuroscience Program

Background: Amyloid PET (aPET) scans provide in vivo evidence of Alzheimer's Disease (AD) however, due to high cost and limited insurance coverage, they are still an impractical for clinical settings. Event Related Potentials (ERPs) are inexpensive, and non-invasive method to measure brain function that may allow an alternative method for detection of AD pathology. We investigated whether ERPs are predictive of aPET status in patients with memory complaints.

Methods: Using a seven-electrode rig in 67 older veterans from a memory disorders clinic completed a three-tone auditory oddball task followed by a standard neuropsychological battery.

Independent t-tests were performed to evaluate differences between groups. Bivariate correlations were performed to assess the relationships between measures. Logistic regression models were used to evaluate potential predictors of amyloid PET status.

Results: Target tone P200 Area under the curve (AUC) at the Cz electrode was greater in the aPET positive group ($M=354.4$), compared to the aPET negative group ($M=157.6$, $p<.05$). P200 target amplitude ($p<.05$) and P200 standard latency ($p<.05$) were significant predictors of amyloid beta positivity. P200 target amplitude had an AUC of 0.66 ($p<.05$) and P200 standard latency had a AUC of 0.73 ($p<.005$) using an ROC analysis.

Conclusions: The P200 response predicts amyloid beta pathology in patients from a memory disorders clinic. Other have shown that P200 represents higher-order processing of sensory information. Posterior synaptic dysfunction in early AD could result in abnormally elevated levels of compensatory cortical activation in the parietal lobe, resulting in increases in P200 amplitude among AD patients.

Department of Biochemistry

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
Margaret Downs (27)
Joseph Kern (*)
Adeline Matschulat (1**)
Jarrod Moore (15***)
Anthony Spinella (5)

27

PROTEOMIC CHANGES TO THE ANTERIOR CEREBRAL ARTERY IN ALZHEIMER'S DISEASE

M. Downs, X. Liu, Y. Zhang, and J. Zaia

Department of Biochemistry

Alzheimer's disease (AD) is the most common cause of dementia. In addition to the hallmark amyloid-β plaques and neurofibrillary tangles, AD is associated with structural vascular abnormalities and aberrant angiogenesis. Biomechanical changes including stiffening and reduced compliance occur in the anterior cerebral artery (ACA). Proteoglycans (PGs) and glycosaminoglycans (GAGs) are crucial for maintenance of mechanical properties of arteries. To better understand their roles, we investigated the proteome and glycome of the ACA in pathological AD and control arteries. Proteins and GAGs were digested from artery rings from AD (n=6, Braak stages V-VI) and control (n=5, Braak stages 0-II) groups using on-slide digestion and analyzed using LC-MS/MS. Ten proteins were found to be significantly differentially expressed (FDR < 0.05) in AD ACAs relative to controls. The expression levels of several members of the collagen family were increased in AD, impacting GAG binding and assembly of the vascular network. Two myosin components were underexpressed in AD, which may affect the smooth muscle contraction of the ACA. Gene set enrichment analysis also demonstrated an under-expression of the structural constituent of muscle gene set (GO:0008307). The proteomic alterations of ACAs observed in AD are indicative of extracellular matrix and smooth muscle dysfunction, which are consistent with the previously observed biomechanical changes. This is the first study to connect proteomic alterations to biomechanical changes in the human cerebrovasculature in AD.

*

HIPPO PATHWAY INACTIVATION DRIVES BASAL-LIKE BREAST CANCER DEVELOPMENT*

J. G. Kern[1], A. Tilston-Lunel[1], A. Federico[2], G. Peppler[1], B. Ning[2], N. Cheng[1], E. Stampouloglou[1], M. Lenburg[2,3], J. Beane[2,3], S. Monti[2], and X. Varelas[1]

[1] Department of Biochemistry
[2] Department of Medicine, Computational Biomedicine
[3] Bioinformatics Program

Basal-like breast cancers, a breast cancer subtype with poor treatment options, exhibit alterations in epithelial and stromal cell populations that contribute to aggressive pathogenesis. These cancers are thought to originate from luminal mammary epithelial cells that undergo plasticity to acquire basal-like and invasive properties upon transformation and metastasis. Identifying mechanisms that promote this luminal-basal plasticity may allow the development of novel treatments for this disease. The Hippo pathway regulates cellular fate in many contexts. Using genetic mouse models, we found that conditional co-deletion of the Hippo pathway kinases LATS1 and LATS2 (LATS1/2) in mouse luminal mammary epithelial cells promotes rapid proliferation and acquisition of basal-like traits that are mediated by the downstream transcriptional effectors YAP and TAZ (YAP/TAZ), leading to the development of basal-like ductal carcinomas with metastatic potential. Bulk and single cell RNAseq analyses demonstrated that LATS1/2-cnull luminal cells adopt gene expression signatures that correlate with mammary basal cells and human basal-like breast cancers, indicating luminal-to-basal plasticity. Further, single-cell RNAseq analyses revealed distinct stromal remodeling, including the presence of fibroblast populations enriched for the expression of genes encoding extracellular matrix (ECM) proteins observed in LATS1/2-cnull mammary glands. Our study highlights the Hippo pathway as an important regulator of mammary gland homeostasis *in vivo* and demonstrates that dysregulation of Hippo signaling promotes autonomous and non-autonomous cues leading to basal-like breast cancer progression.

1**

ROLES FOR HIPPO PATHWAY KINASES LATS1/2 IN EPITHELIAL-IMMUNE HOMEOSTASIS IN THE LUNG

A. Matschulat, A. Tilston-Lunel, C. Cheng, J. Hicks-Berthet, J. Kern, N. Kingston, E. Stampouloglou, A. Shenoy, N. Etesami, J. Mizgerd, B. Ning, M. Lenburg, J. Beane, B. Tilton, A. Belkina, and X. Varelas

Department of Biochemistry

The LATS1 and LATS2 kinases are major regulators of the transcriptional effectors YAP and TAZ, and together function as effectors of Hippo pathway signaling that control essential aspects of organ development and tissue homeostasis. In this study we set out to understand the roles of LATS1/2-YAP/TAZ signaling in the lung, focusing on functions in the distinct cell types of the airway epithelium. Interestingly, we found conditional deletion of LATS1/2 in CC10+ve secretory airway cells results in dramatic lung inflammation that severely compromises lung function. Airway epithelial cells with LATS1/2 deletion acquire a thin squamous morphology that is associated with the onset of a AT1-like

gene expression program. Single cell RNA-sequencing and flow cytometry analysis of LATS1/2 deleted lung have revealed distinct immune cell changes, mirroring aspects of interstitial lung disease. Receptor-ligand mapping signaling has highlighted signals initiated by LATS1/2-deleted cells that recruit innate immune cells that drive inflammation. Our study offers regulatory knowledge into lung epithelial homeostasis and identifies epithelial-immune crosstalk that may contribute to severe lung inflammation observed in disease.

15***

MULTI-OMICS ANALYSIS OF ENGINEERED HYPERTROPHIC CARDIOMYOPATHY TISSUE REVEALS ALTERED MECHANISMS IN MITOCHONDRIAL DYNAMICS AND EXCITATION CONTRACTION COUPLING

J. Moore, C. Chen, A. Emili, and J. Ewoldt

Department of Biochemistry

Hypertrophic cardiomyopathy (HCM) is one of the most common inherited cardiomyopathies, and is a leading cause of sudden cardiac death in young adults. HCM is characterized by asymmetric left ventricular thickening, pronounced changes to systolic and diastolic function, and fibrosis. Despite the discovery of numerous sarcomeric protein mutations, the connection between mutagenic changes to the contractile machinery and the observed clinical phenotypes remains sparse.

Quantitative LC/MS analysis is apt to identify disease-related signaling mechanisms in cardiac models. Exploratory proteomic experiments with patient and mouse samples have found HCM specific alterations, including pathways associated with calcium signaling and energy production. Equally, advances in HCM disease modeling allows researchers to generate substantial numbers of mutant cardiomyocytes for global analysis.

In order to study HCM specific alterations, we utilized a model of HCM composed of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) harboring a mutation in myosin heavy chain beta ($MYH7^{R403Q+/-}$) protein, one of the most common genetic loci of HCM. We performed a multi-omics (proteomics, phosphoproteomics, and metabolomics) profiling of $MYH7^{R403Q+/-}$ hiPSC-CMs to discover altered pathways in mitochondria function and morphology, as well as phosphosite level regulation of calcium handling. To this end, we found an HCM specific phosphorylation pattern on sarcoplasmic reticulum related proteins; moreover, we found key proteins regulating mitochondrial fission and fusion and downregulation of mitochondrial DNA (mtDNA) response proteins.

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DEFINING AN AGE-ASSOCIATED, IMMUNE-EVASIVE TUMOR POPULATION IN ORAL SQUAMOUS CELL CARCINOMA

A. Spinella[1], A. Tilston-Lunel[1], S. Monti[2], M. Kukuruzinska[3], and X. Varelas[1]

[1] Department of Biochemistry
[2] Department of Medicine, Section of Computational Biomedicine
[3] Department of Translational Dental Medicine, Henry M. Goldman School of Dental Medicine

Head and neck cancer squamous cell carcinomas (HNSCC) are the seventh most common cancers globally with poor survival rates and largely ineffective pharmacological treatments. While older age is associated with worse five-year survival for HNSCC, most preclinical cancer studies focus on outcomes in young animals, potentially contributing to our poor understanding and limited treatment strategies for the disease. To test the influence of age on HNSCC, we used syngeneic orthotopic xenograft models of HNSCC, performing hemi-lingual injections of 4MOSC1 and DMBA-MOC1 cells into young (8-14-week-old) and old (75-100-week-old) C57BL/6 mice. We tracked tumor growth, performed scRNA-seq, flow cytometry, and histological assays on isolated xenograft tissues. We observed more rapid tumor growth in old animals with xenografts exhibiting reduced CD4+ and CD8+ lymphocytic infiltration and elevated matrix deposition. Aged tumors also exhibited a transcriptionally-distinct, immune evasive tumor cell population associated with elevated levels of the mechanosensitive transcriptional regulator Yes-associated protein (YAP) and Runt-related transcription factor 1 (RUNX1), a DNA-binding transcription factor that has been implicated as a biomarker of worse HNSCC survival. Based on our observations we hypothesize that the aged oral microenvironment is permissive to tumorigenesis in part due to extracellular matrix dynamics that drive YAP and RUNX1 activity that cooperatively promote pro-tumorigenic transcriptional alterations.

Program in Genetics and Genomics

NOTE: Prize winners are noted in the following list of Abstracts by:

*1st Prize, **2nd Prize

The accompanying number indicates each abstract's poster board.

Participants
Jiaji Chen (17**)
Megan Snyder (*)

17**

3D SPATIAL OMIC PROFILING TO UNRAVEL TNBC

J. G. Chen[1], E. Kelley[1], A. Ilinski[2], N. Modanlo[2], D. Li[2], M. Cassidy[3], K. Mahdaviani[1,2], N. Ko[1], and R. Dries[1,4]

Program in Genetics and Genomics
[1] Department of Hematology/Oncology
[2] Translational Research Core
[3] Department of Surgery
[4] Division of Computational Biomedicine

Patient tissue samples are immensely important to research, as they are the main window through which we can peer at disease function *in situ*. They house a wealth of information about the disease, both intrinsic and extrinsic. Additionally, with the advent of spatial transcriptomic technologies, both their expression profiles and the spatial context of where that expression came from across continuous slices of tissue can now be gleaned. However, in some cases, these all-important samples can be vanishingly rare. Triple negative breast cancer (TNBC) is one such example of a disease that could benefit greatly from spatial transcriptomic analysis but has precious few tissue samples. Due to the aggressiveness of TNBC, patients start treatment soon after diagnosis. To obfuscation from apoptotic signals, the only tissues available for spatial transcriptomic analysis are the initial 1.6mm diameter biopsies taken for diagnostic purposes. In order to maximize the data that can be extracted from such a limited and rare sample, we sampled more slices. We created a digital 3D biopsy from a single TNBC biopsy by serially sectioning a total of 8 slices onto a spatial transcriptomics slide (10X Genomics Visium). Using the paired H&E staining images, the spatial information was then aligned into a single continuous 3D dataset. Additionally, with this transcriptomic base to build upon, additional stainings of interest both proteomic and architectural are planned to be created from adjacent slides to the spatial, creating a truly multi-omic 3D representation of the tissue.

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MALIGNANT CELL EXPRESSION OF THE ARYL HYDROCARBON RECEPTOR INDUCES PD-L1 AND IMMUNOSUPPRESSION IN MODELS OF ORAL AND LUNG CANCER*

M. Snyder, J. Kenison-White, B. Lara, D. Sherr, Z. Wang, and K. Yang

Program in Genetics and Genomics

Immunotherapy has shown dramatic results in treating cancer but only in a minority of patients, so there is unmet need to understand the regulation of immunosuppression in tumors. We have shown that the aryl hydrocarbon receptor (AhR) is a central player in regulating immune checkpoints in oral squamous cell carcinoma (OSCC) and may play a similar role in lung cancer. Orthotopic transplant of mouse AhR-knockout (KO) OSCC (MOC1) cells led to complete rejection of tumor formation coupled with increased T cell signaling in tongues, whereas wild type (WT) OSCC challenge led to tumor formation and upregulation of T cell exhaustion pathways and checkpoint molecules. Similar patterns of AhR-facilitated immune protection appear in our lung cancer model. On average, mice transplanted subcutaneously with $CMT167^{AhR\text{-}KO}$ lung adenocarcinoma cells survive up to twice as long as their WT-challenged counterparts; up to 50% of $CMT167^{AhR\ KO}$-transplanted mice never grow tumor and also were partially protected from challenge with $CMT167^{WT}$ cells. $CMT167^{WT}$-transplanted mice exhibited decreased T cell trafficking to tumors while the proportion of $PD1^+$, $CTLA4^+$ and $Lag3^+$ $CD4^+$ and $CD8^+$ T cells were significantly lower in $CMT167^{AhR\text{-}KO}$ tumors. AhR^{KO} in both cell lines decreases PD-L1 expression by 50%, suggesting a mechanism of immune escape by WT tumors. Thus, the immunological protection following transplantation of both AhR^{KO} CMT167 and MOC1 cells suggests downregulation of PD-L1 on malignant cells is at least partially responsible for immune enhancement in AhR^{KO} cells and supports AhR's potential as a viable immunotherapeutic target in at least two cancers.

Graduate Program for Neuroscience

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
Lucas Carstensen (26***)
Kaitlyn Dorst (21)
William Lynch (40)
Thomas Morin (*)
Shen Ning (34**)
Ryan Senne (21)

26***

A RATE CODE OF ENVIRONMENTAL ALTERATION AND HIGH-RESOLUTION 3D POSE TRACKING DURING BEHAVIOR

L. Carstensen, A. Alexander, W. Chapman, Y. Guo, J. Lee, M. Paetl, M. Betke, and M. Hasselmo

Graduate Program for Neuroscience

Most complex behavior requires flexible and efficient spatial navigation, and boundaries and landmarks are prevalent and influence how we navigate and remember familiar locations. The retrosplenial cortex (RSC) is an area that is interconnected with regions of the brain that display spatial correlates such as the hippocampal formation (HPC). Studies in both rodents and humans show these regions can encode the past or present location of objects and position in the environment. For these reasons and the connectivity of RSC, it is important to determine how neurons in RSC represent available cues such as objects or boundaries and their relationship to the local environment. We performed electrophysiological recordings in RSC while rats foraged in arenas in which the local environment was altered. We report RSC neurons display systematic changes in mean firing rate responding to alterations of the environment. These alterations include the arena boundaries rotating, changing size or shape, or an object being introduced. This distinguishes RSC as a region important in orientation, anchoring, and mapping features of environments. Furthermore, analysis of complex behavior requires accurate tracking of the 3D pose of an animal. While most neurophysiological studies track 1-2 points, we expand upon that by tracking ten key points simultaneously from six different viewpoints, in three different modalities: infrared, depth, and RGB video.

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VISUALIZATION AND MODULATION OF HIPPOCAMPAL ENGRAM-DRIVEN NETWORKS

K. E. Dorst[1,*], R. A. Senne[2,*], J. H. Bladon[1], A. Diep[2], O. P. McKissick[2], S. Skelton[2], and S. Ramirez[2,3]

[1] Graduate Program for Neuroscience
[2] Department of Psychological and Brain Sciences
[3] Corresponding author (dvsteve@bu.edu)
[*]These authors contributed equally to the work

Animals have developed a repertoire of defensive behaviors to avoid predators and other noxious stimuli. The successful use of defensive behaviors depends on both the external context and internal state of the animal. Moreover, the outward expression of defensive behaviors during episodes of cognitive dysfunction can be triggered by the unwarranted recollection of a fearful event, which is a hallmark symptom of PTSD. Yet, how cells that encode a given fear memory modulate downstream neural systems to properly gate defensive behaviors is unknown. To address this, we use activity-dependent viral labeling strategies to leverage optical control over cell populations (i.e., an engram ensemble) in the dentate gyrus (DG) that encodes the information of a fearful experience. Here, we optogenetically reactivate a fear engram under various environment sizes to test for its capacity to drive a variety of defensive behaviors based on spatial demands placed on the animal. We found an inverse relationship in the amount of light-induced freezing behavior and the environment size: mice froze during fear engram reactivation in a small environment, but not in a large arena. This suggests alterations in downstream activity within the brain to modulate defensive behavior. Our current work investigates brainwide correlations in cFos expression using network analysis to identify putative hub regions that could mediate these state-dependent alterations in behavioral strategies. By identifying and manipulating areas supporting memory function, it is thereby possible to resolve systems-level biological mechanisms mediating memory's capacity to modulate maladaptive behavioral states.

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THE METHAMPHETAMINE-INDUCED HNRNP H TARGETOME IDENTIFIES CACNA2D2 AS A DOWNSTREAM MECHANISTIC TARGET UNDERLYING BEHAVIOR: PHARMACOLOGICAL VALIDATION WITH PREGABALIN

W. Lynch, P. Ash, R. Babbs, J. Beierle, C. Bryant, M. Chen, J. Cox, J. Dougherty, W. Johnson, A. Kandola, J. Kelliher, M. Rieger, Q. Ruan, K. Szumlinski, B. Wolozin, and E. Yao

Graduate Program for Neuroscience

Misuse of psychostimulants including methamphetamine **(MA)** is a growing public health crisis with no current FDA-approved treatments, illustrating a critical need to understand the neurobiological mechanisms. Certain MA behaviors are heritable and thus identifying causal genetic factors can elucidate novel targets and mechanisms that inform new therapeutics. Our lab previously identified *Hnrnph1*, encoding the RNA-binding protein hnRNP H1 **(H1)** as a quantitative trait gene underlying MA stimulant sensitivity. However, the mechanisms by which *Hnrnph1* regulates MA behaviors remain unclear. We identified *Cacna2d2*, encoding the voltage-gated calcium channel subunit alpha-2-delta-2, as a candidate downstream target by which H1 influences MA locomotor stimulant sensitivity. We observed reduced mRNA at the *Cacna2d2* 3'UTR (where H1 binds) in H1-mutant mice treated with MA compared to

wildtypes, suggesting differential H1 binding affects alpha-2-delta-2 mRNA expression. We subsequently pretreated mice with the anti-epileptic drug pregabalin **(PGB)**, which binds selectively to the alpha-2-delta-2/alpha-2-delta-1 subunits to inhibit presynaptic calcium influx. PGB pretreatment (30 mg/kg, i.p.) reduced acute MA-induced locomotion in female wildtypes, but not female mutants or males of either genotype, without altering locomotion on its own. Furthermore, PGB pretreatment lowered acquisition of MA locomotor sensitization independent of genotype. mRNA and protein quantification suggest PGB pretreatment does not actively alter alpha-2-delta-2 expression levels regardless of main treatment (MA vs. saline). Together, our results suggest the alpha-2-delta-2 subunit causally regulates MA locomotor sensitivity via differential hnRNP H1 binding, while also highlighting PGB as a potential therapeutic for modulating MA stimulant properties.

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FUNCTIONAL RECONFIGURATION OF A TASK-ACTIVE FRONTOPARIETAL CONTROL NETWORK FACILITATES ABSTRACT REASONING*

T. Morin, K. Isenburg, W. Ma, K. Moore, and C. Stern

Graduate Program for Neuroscience

While the brain's functional network structure is largely conserved between resting and task states, small but significant changes in functional connectivity support complex cognition. In this study, we used fMRI to investigate the activation patterns and functional connectivity of brain networks associated with both resting state and an abstract reasoning task. We found that frontoparietal networks, including the cognitive control and dorsal attention networks, were significantly activated during abstract reasoning. We observed that these same task-active regions flexibly altered their functional connectivity when transitioning from the resting state to the abstract reasoning task. Conversely, we observed a stable network core of regions in default and somatomotor networks that was maintained across both resting and task states. We propose that regionally specific changes in functional connectivity put the brain in a "task-ready" state, facilitating efficient task-based activation.

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DEVELOPING MAGNETIC NANOPARTICLES FOR THERAPEUTIC ANTIBODY DELIVERY IN ALZHEIMER'S DISEASE

S. Ning, J. Park, C. Teves, T. Moore, A. Rompala, I. Kim, D. Capen, S. Patel, S. H. Choi, R. Tanzi, and D. Y. Kim.

Graduate Program for Neuroscience

Multiple Alzheimer's disease (AD) clinical trials target pathogenic amyloid-β (Aβ) species using therapeutic anti-Aβ antibodies. However, recent clinical trials demonstrate immediate need for the development of new therapeutic approaches to improve efficacy and reduce passive antibody infusion side effects. The objective of this work is to develop superparamagnetic iron oxide nanoparticles (SPIONs) conjugated with anti-Aβ antibodies to improve upon existing Alzheimer's disease therapeutics. We combined SPIONs with Aβantibodies to develop a therapeutic methodology for rapid and robust

removal of Aβ aggregation using an external magnetic force. SPIONs conjugated with anti-Aβ antibodies selectively bind to Aβ peptides and aggregated Aβ species in our 3D human neural cell culture model of AD. We also used a vertical triculture model of AD to test the effects of alternating magnetic field on microglia Aβ phagocytosis. Application of this superparamagnetic iron oxide nanoparticles immunotherapy in our 3D human neural cell culture model of AD, followed by rapid removal of SPION-Aβ complex by an external magnet force, efficiently decreased soluble and insoluble Aβ species and accumulation of pathogenic phosphorylated tau species. Furthermore, nanoparticles and alternating magnetic field stimulation in a triculture model of AD showed increased Aβ phagocytosis by microglia cells. In 5XFAD mice, we show improved antibody delivery and reduction in Aβ load. Our results demonstrate the therapeutic potential of targeted nanotechnology in reducing both Aβ accumulation and tau pathology in a 3D human neural cell culture model of AD and in the 5XFAD mouse model of AD.

Department of Microbiology

Participants

Elizabeth Chavez (10)
Devin Kenney (7)
Jonathan Kilroy (19)
Victoria Kleiner (39)
Aoife O'Connell (7)
Stephen Ross (37)
Ellen Lee Suder (11)
Jacquelyn Turcinovic (7)

10

IDENTIFICATION OF FLAVIVIRUS RNA ELEMENTS MEDIATING ESCAPE FROM CELL-INTRINSIC IMMUNITY

E. Chavez, M. Matsuo, and F. Douam

Department of Microbiology

Flaviviruses have developed an elaborate panel of mechanisms to inhibit the induction of type I interferon-mediated antiviral signaling. These mechanisms are required to overcome systemic innate immunity and enable extensive replication within tissues. Although escape of cell-intrinsic immunity mediated by flavivirus proteins has been extensively characterized, it is still unclear how specific RNA elements within the flavivirus coding genome contribute to immune escape. To investigate the role of flavivirus RNA elements in escaping cell-intrinsic immunity, we used a bicistronic replicon derived from yellow fever virus (YFV) vaccine (17D) in which sequences encoding for 17D structural proteins have been replaced with a secreted luciferase coding gene. Using this replicon, we generated mutants in which synonymous mutations that disrupted RNA elements were introduced in the non-structural protein coding regions of the genome. Upon transfection into human hepatoma cells, disruption of RNA elements within two small segments (EL1 and EL2) of the 17D coding genome significantly impaired viral RNA replication without compromising intrinsic replication competency. Importantly, interferon-stimulated gene expression was higher in cells transfected with this mutant than in cells transfected with a wild-type or a replication-defective YFV-17D replicon, suggesting that EL1 and EL2 contain RNA elements promoting evasion from cell-intrinsic immunity. Our ongoing work aims to identify sequence determinants in EL1 and EL2 mediating immune escape, as well as further elucidating the mechanism mediated by these elements. Beyond its potential to expand our understanding of flavivirus immune escape, this work will open avenues for enhancing the potency of mRNA-based-therapies and vaccines.

CD4+ CD163+ INTERSTITIAL INFLAMMATORY MACROPHAGES DRIVE SARS-COV-2 CLEARANCE IN HUMAN LUNG TISSUE FOLLOWING INFECTION

D. J. Kenney[1,2,12], A. K. O'Connell[1,2,12], J. Turcinovic[2,3,12], P. Montanaro[2,4], S. Al Abdullatif[5,6], H. P. Gertje[2,4], A. E. Tseng[2,4], A. Sheikh[1,2], M. Ericsson[7], B. R. Huber[8], J. D. Campbell[5,6], R. B. Corley[1,2], J. H. Connor[1,2], V. Vrbanac[2,4], C. Harly[9,10,12], N. A. Crossland[2,4,13], A. B. Balazs[11,13*] and F. Douam[1,2,13*]

[1] Department of Microbiology
[2] National Emerging Infectious Diseases Laboratories
[3] Bioinformatics Program
[4] Department of Pathology and Laboratory Medicine
[5] Single Cell RNA Sequencing Core
[6] Department of Medicine
[7] Electron Microscopy Core Facility
[8] Department of Neurology
[9] Université de Nantes, INSERM, CNRS, CRCINA, Nantes, France
[10] LabEx IGO 'Immunotherapy, Graft, Oncology', Nantes, France.
[11]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA.
[12] These authors contributed equally to the work.
[13] These authors contributed equally to the work.
[*] Lead Contacts

While animal models and patient studies have been instrumental to capture cellular and molecular correlates of SARS-CoV-2 pathogenesis, the mechanisms driving lung tissue protection during SARS-CoV-2 infection in human remain elusive. This knowledge gap mostly stems from the lack of tractable animal models recapitulating protective human pulmonary immune responses during infection. Here, we report that mice co-engrafted with human fetal lung xenograft (fLX), human fetal thymus and liver, as well as human hematopoietic stem cells (BLT-L mice) are readily susceptible to SARS-CoV-2 infection and mount effective human immune responses against this virus. Following direct, subcutaneous viral inoculation into fLX, BLT-L mice were able to successfully clear fLX of virus while mounting tissue repair mechanisms by 12 days post inoculation. Viral clearance associated with significant infiltration and activation of CD4+ CD163+ interstitial inflammatory macrophages (CD163+ IM) at day 2 post infection. Interestingly, antibody mediated-CD4+ cell depletion associated with a dramatic reduction of interstitial macrophages in fLX post infection, and abrogated viral clearance. Neither effector T cell nor neutralizing responses could be detected at any time points in infected BLT-L mice prior to and after viral clearance, underscoring a critical role for CD4+ CD163+ IM in driving lung tissue protection from SARS-CoV-2 infection. Taken together, our work provides the first experimental evidence that CD4+ CD163+ IM are critical sentinels protecting the human lung against SARS-CoV-2 infection.

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EVALUATING THE IMPACT OF DEFECTIVE VIRUSES ON IMMUNE CELL FUNCTION

J. Kilroy; V. Dominguez; A. Henderson; and J. Kuniholm

Department of Microbiology

HIV persistence is driven by a subset of cells harboring transcriptionally repressed latent HIV that contributes to rebound upon treatment interruption presenting a challenge to HIV cure. However, examination of the persistent viral sequences has shown that the reservoir consists of mostly defective viruses with point mutations, frame shifts, inversions, and deletions which would limit productive HIV-1 transcription. Furthermore, even in the presence of antiretroviral therapy and limited HIV transcription, PLWH demonstrate chronic comorbidities of the central nervous system, heart, and general inflammaging. It is unknown what drives the dysregulated inflammation in PLWH. We hypothesize that the expression of defective proviruses activate innate immune activities in T cells and myeloid cells to perpetuate inflammation. This hypothesis is supported by previous work where we demonstrated expression of RNAs and proteins from defective proviruses driven by an intragenic promoter. To further investigate the function of these defective proviruses, we are employing CRISPR-Cas9 to engineer cells harboring defective HIV proviral genomes lacking 5' LTRs. These cells are being used to characterize the expression of defective proviruses and whether they are influencing IFN type I responses and the expression of inflammatory cytokines. Overall, these preliminary studies provide a foundation in understanding mechanisms that contribute to HIV-1 mediated immune dysfunction and pathogenesis.

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DISTINCTIVE FEATURES OF THE RESPIRATORY SYNCYTIAL VIRUS PRIMING LOOP COMPARED TO OTHER NON-SEGMENTED NEGATIVE STRAND RNA VIRUSES

V. A. Kleiner, P. O. Byrnec, T. N. Cressey, R. Fearns, J. McLellan, E. Mühlberger**,** S. L. Noton, and A. M. Shareef

Department of Microbiology

De novo initiation by viral RNA-dependent RNA polymerases often requires a polymerase priming residue, located within a priming loop, to stabilize the initiating NTPs. Polymerase structures from three different non-segmented negative strand RNA virus (nsNSV) families revealed putative priming loops in different conformations, and an aromatic priming residue has been identified in the rhabdovirus polymerase. In a previous study of the respiratory syncytial virus (RSV) polymerase, we found that Tyr1276, the L protein aromatic amino acid residue that most closely aligns with the rhabdovirus priming residue, is not required for RNA synthesis but two nearby residues, Pro1261 and Trp1262, were required. In this study, we examined the roles of Pro1261 and Trp1262 in RNA synthesis initiation. Biochemical studies showed that substitution of Pro1261 inhibited RNA synthesis initiation without inhibiting back-priming, indicating a defect in initiation. Biochemical and minigenome experiments showed that the initiation defect incurred by a P1261A substitution could be rescued by factors that would be expected to increase the stability of the initiation complex, specifically increased NTP concentration, manganese, and a more efficient promoter sequence. These findings indicate that Pro1261 of the RSV L protein plays a role in initiation, most likely in stabilizing the initiation complex. However, we found that substitution of the corresponding proline residue in a filovirus polymerase had no effect on RNA synthesis initiation or elongation. These results indicate that despite similarities between the nsNSV polymerases, there are differences in the features required for RNA synthesis initiation.

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EBOLA VIRUS LEADER RNAS INFORM NP-DEPENDENT MODEL FOR TRANSCRIPTION-REPLICATION TRANSITION DURING INFECTION

S. Ross[1,2,3], D. Cifuentes, and E. Mühlberger[1,2]

[1] Department of Microbiology
[2] National Emerging Infectious Diseases Laboratories
[3] Department of Biochemistry

Ebola virus (EBOV) is a non-segmented, negative sense RNA virus which causes severe hemorrhagic fever in humans. EBOV encodes its own RNA-dependent RNA polymerase complex to both transcribe and replicate its genome. However, the transition from transcription to genome replication is not well understood. For many NNS RNA viruses, including EBOV, it has been reported that small RNAs complementary to the 3' leader region are synthesized during infection. EBOV leader RNAs are approximately 72 nucleotides. We posit these leader RNAs direct transcription initiation in which leader RNA release allows for RdRp recognition of the first gene start sequence. However, there is no termination sequence within the leader to allow for leader RNA release. We propose that leader RNA synthesis, release, and transcription initiation is determined by the levels of nucleoprotein (NP) expression and subsequent leader RNA encapsidation into a ring-like structure. Using EBOV minigenome systems, we have demonstrated that leader RNA expression directly correlates with reporter gene activity, suggesting a link between leader RNA synthesis and reporter transcription. Furthermore, both reporter gene expression and leader RNA synthesis are dependent on the levels of NP expressed within the system. Current work intends to determine whether EBOV leader RNA is encapsidated like VSV leader RNA. This encapsidation could serve as a potential structure-encoded stop which terminates leader RNA synthesis. Ultimately, we aim to explain how the levels of NP expressed during EBOV infection regulate the balance between transcription and replication.

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TRANSCRIPTION AND BEYOND: ELUCIDATING THE FUNCTIONS OF MARBURG VIRUS VP30

E. L. Suder and E. Mühlberger

Department of Microbiology

The filovirus phosphoprotein VP30 serves as a necessary transcriptional activator for Ebola virus (EBOV), while in contrast it is a dispensable enhancer of Marburg virus (MARV) transcription when examined in minigenome systems; however, rescue of infectious virus from any full-length filovirus genome requires VP30 regardless of genus. While dynamic phosphorylation of MARV VP30 impacts viral transcription efficiency, no mechanism has been identified for the necessity of MARV VP30 in viral rescue, and its role in authentic viral replication remains largely speculative. Here we present data obtained with MARV multicistronic minigenome assays, transcription- and replication-competent virus-like particle (trVLP) systems, and targeted protein degradation experiments for discrete investigation of potential MARV VP30 functions, including transcriptional elongation and re-initiation and viral packaging. Our data show that reporter genes inserted downstream of the first gene position in

multicistronic minigenomes are efficiently expressed in the absence of VP30, suggesting MARV VP30 is not needed for transcription re-initiation. We also find that infectious trVLP formation occurs only in the presence of VP30 supplied in trans, potentially implicating the protein in particle formation or in primary transcription. Due to the necessity of VP30 for EBOV transcription initiation, which prevents any further steps from occurring in the absence of VP30 in any EBOV system, our established MARV systems provide a means by which to investigate subsequent VP30 functions in the viral replication cycle and broaden our understanding of VP30's role for filoviruses as a whole.

Graduate Program in Molecular and Translational Medicine

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

Senegal Carty (33)
Elissa Everton (9**)
Stacy Jankowski (36)
Yu Liu (42)
Liang (Martin) Ma (25)
Emilie Mausser (13)
Elim Na (8***)
Ellena Nador (12)
Yuhan Qiu (43)
Anna Smith (3)
Anthony Yeung (*)

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PULMONARY LYMPHATIC VASCULATURE REMODELING IN RESPONSE TO INFLUENZA INFECTION

S, Carty, E. Crossey, F. Shao, A. Ysasi, M. Zeng, J. Yuan, J. Henao-Vasquez, A. Hinds, A. Fine, and M. Jones

Graduate Program in Molecular and Translational Medicine

In inflamed tissue, the lymphatic vasculature's role is vital. Lymphatic vessels drain excess fluid to limit edema, transport immune cells and antigen to lymph nodes and shape immune responses via signaling between lymphatic endothelial cells (LECs) and leukocytes. The pulmonary lymphatics frequently respond to respiratory tract inflammation, of which influenza is a common and virulent cause. However, our understanding of this response is limited. We hypothesize that in influenza, lymphatic vessels undergo remodeling to improve viral clearance and modify immune cell activity. To determine whether lymphangiogenesis, or lymphatic vessel growth, occurs in the influenza-infected lung, we performed immunohistochemistry to detect PROX1, the master regulator of LEC differentiation, in murine lung tissue sections, then enumerated the LEC nuclei. EdU incorporation was measured to determine the role of LEC proliferation in flu-induced lymphatic expansion. We also investigated whether an LEC progenitor contributed to lymphangiogenesis by fluorescently labelling LECs before infection, then comparing the proportion of labelled LECs in flu-infected and control mice. Additionally, we are using fluorescence-activated nuclei sorting (FANS) to isolate LEC nuclei and enable unbiased sampling for transcriptional profiling via single nuclei RNA sequencing (snRNAseq). Here we show that, in addition to lymphatic dilation, influenza infection induces dramatic pulmonary lymphangiogenesis, driven mainly by LEC proliferation. Using tamoxifen-inducible GFP labelling of PROX1-positive nuclei and FANS, we have obtained high-quality yields of isolated LEC nuclei. In future, we will perform snRNAseq to determine whether pulmonary LEC subtypes arise in the context of influenza infection, and elucidate their roles in immune system regulation.

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ACTIVATION OF GROWTH HORMONE PATHWAY TO ACCELERATE RECOVERY FROM ACETAMINOPHEN-INDUCED MURINE LIVER INJURY**

E. Everton[1], M. Del Rio Moreno[2], J. Lindstrom-Vautrin[1], C. Villacorta-Martin[1], N. Pardi[3], R. Kineman[2], and V. Gouon-Evans[1]

Graduate Program in Molecular and Translational Medicine
[1]Center for Regenerative Medicine, Department of Medicine
[2]Department of Medicine, University Chicago at Illinois
[3]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania

Acetaminophen (APAP) is the most commonly used pain reliever in the US, yet APAP overdose is the leading cause of acute liver failure in the developed world. The only available treatments are N-acetyl cysteine (NAC), which has a short window of effectiveness, or liver transplant, presenting a need for alternative therapeutics. This project addresses this issue by deciphering the mechanisms of sexual dimorphism in APAP-induced liver injury and repair, and leveraging it with growth hormone (GH) therapy to accelerate liver recovery in both sexes following APAP overdose. Consistent with the literature, our data support the higher resistance of female mice to APAP, shown by reduced liver necrosis and serum injury markers compared to males. Single-cell RNA sequencing analyses reveal that female hepatocytes express significantly greater levels of GH receptor and GH pathway activation. In harnessing this female-specific mechanism, a single injection of recombinant human GH accelerates liver regeneration in both sexes following severe doses of APAP, exhibited by rapid and significant repair of the liver and promotion of survival, compared to vehicle control and NAC. Importantly, hepatocyte-specific expression of either human GH via delivery of non-integrative lipid nanoparticle-encapsulated mRNA or GH effector Stat5b via transduction of AAV8-TBG-Stat5b also promote liver recovery. Overall, these key data demonstrate a sexually dimorphic liver regenerative advantage in females, which we leveraged by establishing GH as an alternative treatment to accelerate recovery in both sexes; critical in preventing liver failure and liver transplant requirements for APAP overdosed patients.

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SERINE SYNTHESIS AND CELL IDENTITY IN ORAL SQUAMOUS CELL CARCINOMA

S. A. Jankowski; N. Hardy; and M. A. Kukuruzinska

Graduate Program in Molecular and Translational Medicine
Department of Translational Dental Medicine, Henry M. Goldman School of Dental Medicine

Oral squamous cell carcinoma (OSCC) is a devastating malignancy associated with high morbidity, poor survival, and few therapeutic options. OSCC is characterized by heterogeneous cell states, including cancer stem cells (CSCs). Previous studies have shown that cancer cells acquire distinct metabolic adaptations to facilitate tumor development, including abnormal reliance on exogenous serine at the expense of endogenous serine synthesis. A by-product of the serine synthesis pathway, alpha-ketoglutarate, serves as an obligate co-substrate of alpha-ketoglutarate dependent dioxygenases, which function as chromatin-modifying enzymes that demethylate histone marker H3K27me3, thus de-repressing differentiation markers.

In this study, we have aimed to decode the relationship between serine synthesis and cell identity in OSCC. We now show that OSCC cells lines ceased proliferation and changed their morphology from mesenchymal to epithelial under serine starvation conditions. This was associated with a statistically significant increase in the steady-state mRNA and protein levels of the serine synthesis pathway (SSP) enzymes, indicating that OSCC cell lines rely on exogenous serine to maintain their stem cell-like states. Accordingly, upregulation of SSP enzymes in OSCC cell lines was accompanied by an increase in alpha-ketoglutarate concentration which, in turn, was associated with increased steady-state transcript abundance of a differentiation marker, KRT10. These results suggested that serine deprivation promotes the activity by alpha-ketoglutarate dependent dioxgenases to demethylate histone marker H3K27me3. Our findings suggest that a switch from exogenous serine to the endogenous serine biosynthesis pathway promotes OSCC cell differentiation concomitant with the loss of stem cell identity.

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DIVERSITY OF PRX1 STEM/PROGENITOR CELLS AND THEIR REGULATION FOR BONE REGENERATION

Y. Liu, A. Ilinski, L. Gerstenfeld, and B. Bragdon

Graduate Program in Molecular and Translational Medicine
Department of Orthopaedic Surgery

Introduction: The goal of this study is to characterize the skeletal stem cells of Prx1 lineage from different tissues, and to assess the effect of environmental and intrinsic regulations on stem cell differentiation.

Methods: Prx1CreER;R26-*tdTom*;Rag mice were implanted with BMP2 (0-0.5ug) treated sponges within the muscle or on periosteal surface to collect local cells. Then sponges were transferred to either the periosteal surface or muscular pouch of a WT host. For injury response, the sponge was transplanted adjacent to a fracture at time of injury. Bone formation and contribution of Prx1 cells were evaluated radiologically and histologically. To study the intrinsic differences among Prx1 populations, P1 (CD105-CD200+), P2(CD105-CD200-) and P3 (CD105+) from periosteum and muscle were analyzed by bulk-RNAseq and in vitro culture.

Results: Results showed periosteal Prx1 cells with low BMP2 (0.3 - 0.5 μg) induced bone formation when transplanted to the periosteum, while muscle Prx1 cells with BMP2 failed to induce bone. Both the periosteal and muscle derived Prx1 cells with BMP2 could not induce bone formation within the muscle. To confirm muscle derived Prx1 cells possess the osteogenic capacity, 5μg BMP2 was shown to induce bone formation at periosteum and muscle. Under injury conditions, both periosteal and muscle Prx1 cells contributed to the fracture callus without exogenous BMP2. This indicates a possible balance between environmental and intrinsic regulation. The bulk-RNAseq analysis demonstrated Prx1 cells are distinct between the three tissues. The periosteal derived P1 is non-proliferative, enriched for ossification related GO terms, while P2 and P3 are enriched with cell division related GO terms. All three subpopulations of the muscle derived Prx1 cells are non-proliferative.

Conclusions: Our data suggests that the Prx1 derived periosteal and muscle cells are distinct with varying genetic regulation, however both are modulated by the local environment and respond to injury.

REGENERATION OF MOUSE TRACHEAL EPITHELIUM VIA TRANSPLANTATION OF PLURIPOTENT STEM CELL-DERIVED BASAL LIKE CELLS

L. Ma, F. Hawkins, M. Herriges, D. Kotton, J. Landstrom-Vautrin, J. Le Suer, B. Thapa, A. Tilston-Lunel, X. Varelas, C. Villacorta-Martin, and F. Wang

Graduate Program in Molecular and Translational Medicine

Lung diseases resulting from airway epithelial dysfunction such as cystic fibrosis are responsible for significant morbidity and mortality. In vivo engraftment of mutation-corrected, healthy airway stem cells generated exogenously from pluripotent stem cells (PSC) could provide a putative autologous cell-based therapy for airway diseases. To simulate future transplantation approaches for treating recipients without immunosuppression, we sought to develop a "pre-clinical" model by transplanting syngeneic engineered mouse cells into immunocompetent mice.

Mouse PSCs were differentiated into airway basal-like cells (iBCs) via in vitro directed differentiation. RT-qPCR and scRNA-Seq revealed iBC cultures were composed of predominantly by airway basal-like cells and a minor population of secretory-like cells. When transplanted into syngeneic immunocompetent mice following tracheal epithelial injury by polidocanol, iBC-derived cells can be observed more than 300 days post-transplantation. Flow cytometry, immunofluorescence, and scRNA-Seq revealed transplanted cells can contribute to more than 50% of recipients' tracheal epithelium. They maintained their airway epithelial cell identity and expressed both the morphologic and molecular phenotypes of differentiated airway basal, secretory, and ciliated lineages while assuming quiescence. iBC-derived basal cell can participate in injury repair after a second round of polidocanol injury, and iBC-derived ciliated cells can perform normal ciliary movement, suggesting their in vivo function. Overall, our findings suggest that airway basal-like cells generated from PSC can engraft in airway epithelium in vivo. These results set the stage for follow-up human iBC transplantation and disease model rescue experiments, and may serve as a tentative first-step towards potential future regenerative therapy for patients with airway diseases.

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THE FUNCTION OF THE HUMAN CONTRACEPTION ANTIBODY MAY BE ENHANCED BY INTERACTIONS WITH PHAGOCYTIC CELLS, COMPLEMENT, AND CERVICAL MUCUS

E. Mausser, D. Anderson; R. Brosnan, J. Marathe; E. Nador, J. Politch, M. Tjilos, and K. Whaley

Graduate Program in Molecular and Translational Medicine

Despite the many advances in contraceptive technologies, up to 40% of pregnancies worldwide are classified as unintended. To address this issue, we are developing a topical immunocontraceptive film, ZB-06, containing a human anti-sperm antibody, the "Human Contraception Antibody" (HCA). HCA potently agglutinates sperm cells, but its Fc interactions in the female reproductive tract have not been thoroughly examined. In this study we compare the function of HCA in the presence of macrophages, cervical mucus (CM), and complement to HCA-LALAPG, a variant with a mutated Fc region, and HCA-IgGt, a hexameric IgG. PMA-activated U937 cells were used for antibody-dependent cellular phagocytosis (ADCP), and the number of HCA-treated sperm associated with the U937 cells was recorded. Ovulatory CM was loaded into flat capillary tubes and sperm penetration was evaluated with

and without HCA. Sperm immobilization in the presence of HCA and serum complement was compared to heat-inactivated serum. HCA induced significantly more ADCP than HCA-LALAPG. HCA also significantly reduced the penetration of progressively motile sperm into CM compared to both no antibody and HCA-LALAPG. With HCA, sperm also appeared to be trapped in the CM, having flagellar beating without forward motility. When mixed with sperm and human serum, HCA and HCA-IgGt readily immobilized sperm while HCA-LALAPG did not. These findings suggest that the contraceptive potential of HCA may be increased through Fc interactions with phagocytic cells, as well as with mucins and complement proteins in CM, by trapping and possibly killing sperm cells, limiting their progression through the female reproductive tract.

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EPITHELIAL LIF SIGNALING LIMITS LUNG INJURY AND APOPTOSIS DURING PNEUMONIA

E. Na, E. Allen, L. Baird, C. Odom, F. Korkmaz, A. Shenoy, A. Matschulat, M. Jones, D. Kotton, J. Mizgerd, X. Varelas, K. Traber, and L. Quinton

Graduate Program in Molecular and Translational Medicine

Pneumonia is a worldwide public health concern representing a leading burden of disease. Leukemia inhibitory factor (LIF), an IL-6 family cytokine, is crucial for tissue protection during pneumonia. However, cellular targets and signaling networks required for LIF-mediated protection are unknown. C57BL/6J mice received *E. coli* co-instilled with either control IgG or LIF-neutralizing IgG for 6 or 24 hours. GSEA of a microarray conducted on sorted epithelial cells revealed that apoptosis-related pathways were upregulated in the anti-LIF group compared to IgG. TUNEL staining and flow cytometry (Annexin V+, PI-) confirmed that there were more apoptotic cells in anti-LIF compared to IgG. In separate studies, wildtype and EpiLIFR$^{\Delta/\Delta}$ mice (lacking LIFR on lung epithelium) were infected with *E. coli* for 24 hours, and results from these studies indicate a significant increase in lung injury following LIFR deletion. Furthermore, exogenous recombinant murine LIF administration during bacterial challenge in wildtype mice had a modest but significant effect on pneumonia outcome. Single-cell RNA-sequencing of lung cells from C57BL/6J mice treated with IgG saline, IgG *E. coli*, or anti-LIF *E. coli* for 24 hours revealed that ATII cells, endothelial cells, and mesenchymal cells express *Lifr*. Unsupervised clustering showed significant changes in ATII cells during pneumonia, with additional differences following LIF neutralization, However, the direct contributions of LIFR in epithelial cells are still unclear, and future studies will focus on the mechanisms underlying LIF-mediated protection in other cell types during pneumonia, possibly revealing novel targets for clinical intervention in patients with or at risk for lung infections.

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TOPICAL DELIVERY OF SYNTHETIC MRNA EXPRESSING HUMAN CONTRACEPTION ANTIBODY (HCA) TO THE FEMALE REPRODUCTIVE TRACT FOR CONTRACEPTION

E. Nador, D. Vanover, J. Y. Joo, R. Hobbs, F. Villinger, K. Whaley, J. Politch, D. Anderson, P. Santangelo

Graduate Program in Molecular and Translational Medicine

High rates of unintended pregnancies worldwide indicate the need for more acceptable contraceptive options. Human contraception antibody (HCA), an IgG1 monoclonal antibody that agglutinates and immobilizes human sperm, is a promising candidate for nonhormonal immunocontraception in women. Here, we explored an mRNA-based topical delivery method to induce expression of HCA in the female reproductive tract (FRT). The use of synthetic mRNA for contraception is novel and could provide several advantages including: efficiency, reversibility, safety, durability, and cost-effectiveness.

Transfection experiments were conducted *in vitro* using human vaginal epithelial cell lines and a 3D vaginal tissue model and *in vivo* using rhesus macaques. Target cells were transfected with synthetic mRNA strands encoding GPI-anchored or secreted HCA using an atomization device. Vaginal cells were conducive to aerosol mRNA transfection and subsequently produced antibody both *in vitro* and *in vivo*. HCA expression was reversible and did not disrupt cell viability or tissue permeability. Greater than 10 μg/ml of antibody (minimum functional dose) was produced over consecutive days, and the antibody agglutinated sperm. The GPI-anchored HCA demonstrated enhanced retention compared to secreted HCA and may be especially suitable for longer-lasting contraception. Furthermore, preliminary data show that a multivalent variant of HCA assembled with IgM tailpiece can also be expressed in cells from mRNA and has increased potency compared to IgG1 HCA. These data support the further exploration of topically delivered mRNA for the expression of HCA in the FRT as nonhormonal female contraception.

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EXOSOMES PRODUCED BY ADIPOCYTES INDUCE EMT, AND TUMOR METASTASIS, IN BOTH *IN VIVO* AND *IN VITRO* MODELS OF TNBC

Y. Qiu, C. Ross, N. Jafari, M. Kolla, I. Pompa, P. Llevenes, C. Ennis, C. Mazzeo, K. Mahdaviani, N. Ko, and G. Denis

Graduate Program in Molecular and Translational Medicine

Type 2 Diabetes (T2D) is a chronic disease characterized by inflamed adipose tissue. Patients with triple negative breast cancer (TNBC) and comorbid T2D have higher risk of metastasis and shorter survival. However, mechanisms that couple T2D to TNBC outcomes are unknown. Here we hypothesize that exosomes, small vesicles secreted by tumor microenvironment (TME) breast adipocytes, drive epithelial-to-mesenchymal transition (EMT), metastasis in TNBC. Exosomes were purified from conditioned media of 3T3-L1 mature adipocytes, either insulin-sensitive (IS) or insulin-resistant (IR), then characterized and quantified by NanoSight. Murine 4T1 cells, a TNBC model, were treated with exosomes *in vitro* (3 days). For *in vivo* models, mammary fat pads of BALB/c mice were injected with 4T1 cells. Histology and immunohistochemistry detected TME differences (angiogenesis; adipocyte number, size). Metastases in

distant organs were visualized and quantified by clonogenic assay. Exosomal RNAs were profiled by miRNA array to identify potential candidates responsible for driving metastasis. Tumor-bearing mice exhibited more metastasis in exosome-treated groups. In primary tumors, EMT gene transcription and angiogenesis biomarker CD31 were elevated in IR exosome group vs. control and IS exosomes groups. Clonogenic assay of brain metastases showed IR group cells have more mesenchymal morphology. miR-let-7b is highly differentially expressed between IS and IR, and potentially regulates metastasis. IR adipocyte exosomes modify TME, increase EMT and promote metastasis to distant organs, likely through miRNA pathways. We suggest metabolic diseases (e.g T2D) reshape TME, promoting metastasis and decreasing survival. Therefore, TNBC patients with T2D should be closely monitored for metastasis, with metabolic medications considered.

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PRIMARY HEPATOCYTE AND ENGINEERED iPSC-DERIVED HEPATOCYTE-LIKE CELL TRANSPLANTATION TO TREAT ALPHA-1 ANTITRYPSIN DEFICIENCY ASSOCIATED LIVER DISEASE

A. R. Smith, K. Kiwimagi, Y. Tam, N. Pardi, D. Weissman, R. Weiss, V. Gouon-Evans

Graduate Program in Molecular and Translational Medicine

The genetic disease alpha-1 antitrypsin deficiency (AATD) causes AAT protein to misfold, leading to polymerization in hepatocytes, cell death, and cirrhosis. Curing AATD requires replacing diseased hepatocytes with wild type, but donor organs for whole liver transplantation and primary human hepatocytes (PHH) for cell therapy are scarce. Instead, AATD patient induced pluripotent stem cells (iPSC) that have been gene corrected and differentiated could provide an unlimited supply of healthy autologous hepatocyte-like cells (HLC) for transplantation. Currently, factors that limit HLC engraftment in mouse models of liver diseases are not fully understood, but include poor survival, proliferation, and maturation of transplanted cells. Therefore, we hypothesize that stimulating key regenerative pathways in transplanted hepatocytes using hepatocyte growth factor (HGF) and epidermal growth factor (EGF) will improve survival, proliferation, and engraftment of PHHs and HLCs in NSG-PiZ mice, a mouse model recapitulating human AATD liver disease. We have established a safe way to transiently express HGF and EGF in the liver using nonintegrative nucleoside-modified mRNA encapsulated in lipid nanoparticles (mRNA-LNP). Importantly, we show that control PHH engraftment is significantly improved with HGF+EGF mRNA-LNP treatment in NSG-PiZ mice, and transplanted HLC survival is transiently improved with HGF+EGF mRNA-LNP treatment. Ongoing studies aim to (1) engineer HLCs to express physiological levels of receptors for HGF and EGF, (2) engineer HLCs to express higher levels of key hepatocyte maturation factors, and (3) transiently halt host mouse hepatocyte proliferation using P21 mRNA-LNP to further advantage transplanted cell engraftment, all to mitigate AATD associated liver disease.

*

DE-NOVO HEMATOPOIESIS FROM THE FETAL LUNG

A.K. Yeung[1,2], C. Villacorta-Martin[1], J. Lindstrom-Vautrin[1], A. C. Belkina[4,5], K. Vanuytsel[1,2], T. W. Dowrey[1,2], A. B. Ysasi[1,3], V. Vrbanac[6], G. Mostoslavsky[1,2], A. B. Balazs[6], G. J. Murphy[1,2]

Graduate Program in Molecular and Translational Medicine
[1]Center for Regenerative Medicine of Boston University and Boston Medical Center
[2]Section of Hematology and Medical Oncology
[3]Pulmonary Center and Department of Medicine
[4]Flow Cytometry Core Facility
[5]Department of Pathology and Laboratory Medicine
[6]Ragon Institute of MGH, MIT and Harvard, Cambridge, Massachusetts

Hemogenic endothelial cells (HECs) are specialized cells that undergo endothelial to hematopoietic transition (EHT) to give rise to the earliest precursors of hematopoietic progenitors that will eventually sustain hematopoiesis throughout the lifetime of an organism. Although HECs are thought to be primarily limited to the aorta gonad mesonephros (AGM) during early development, EHT has been described in various other hematopoietic organs and embryonic vessels. Though not traditionally seen as a hematopoietic organ, the lung houses many resident hematopoietic cells, aids in platelet biogenesis and is a reservoir for hematopoietic stem and progenitor cells (HSPCs). However, lung HECs have never been described. Here we demonstrate that the fetal lung is a novel source of HECs that have the functional capacity to undergo EHT to produce de novo HSPCs and their resultant progeny. Explant cultures of murine and human fetal lungs display adherent endothelial cells transitioning into floating hematopoietic cells accompanied by the gradual loss of an endothelial signature. Flow cytometric and functional assessment of fetal lung explants showed the production of multipotent HSPCs that expressed the EHT and pre-HSPC markers EPCR, CD41, CD43 and CD44. scRNA-Seq and small molecule modulation demonstrated that fetal lung HECs rely on canonical signaling pathways to undergo EHT, including TGFβ/BMP, Notch and YAP. Collectively, these data strongly support the concept that post-AGM development, functional HECs are present in the fetal lung establishing this location as a potential extramedullary site of de-novo hematopoiesis.

Nutrition and Metabolism Program

NOTE: Prize winners are noted in the following list of Abstracts by:

*1st Prize

The accompanying number indicates each abstract's poster board.

Participants
Ioanna Yiannakou (28)
Mengjie Yuan (*)

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DIETARY PATTERNS AND RISK OF NON-ALCOHOLIC FATTY LIVER DISEASE IN THE FRAMINGHAM STUDIES

I. Yiannakou[1,2], M. R. Singer[1], M. T. Long[3*] & L. L. Moore[1*]

Nutrition and Metabolism Program
[1] Department of Medicine/Preventive Medicine and Epidemiology
[2] Ph.D. in Biomedical Science, Nutrition and Metabolism
[3] Department of Medicine, Section of Gastroenterology

Given that there is no consensus on the pharmacotherapy for non-alcoholic fatty liver disease (NAFLD), dietary interventions are essential preventive measures. However, the optimal diet is not known. We examined the prospective association of various dietary patterns with NAFLD risk and evaluated whether cardiometabolic dysfunction modify these associations in a US community setting. We included participants from the Framingham Heart Study second and third-generation cohorts. Liver fat was assessed with a repeated computed tomography scans using the liver phantom ratio (LPR). Incident NAFLD was defined as LPR ≤0.33 after excluding prevalent NAFLD. Food frequency questionnaires were used to calculate baseline adherence to Dietary Approach to Stop Hypertension (DASH), Mediterranean diet (MeDiet), low-fat diet (LFD), and low-carbohydrate diet (LCD) scores. Modified poison regression models were used to compute risk ratios (RR) and confidence intervals (CI) and adjusted for age, sex, education, lifestyle confounders, and BMI change. Over 6 years, 18.6% of participants developed NAFLD. DASH diet was inversely associated with NAFLD risk, independent of BMI changes (RR: 0.60, 95% CI: 0.42-0.86). Women with the highest adherence to LCD (vs. lowest) had an 89% increased risk of NAFLD (95% CI: 1.16–3.08); these effects were modified by BMI and diabetes. No associations were found in men. LFD or MeDiet scores were not associated with NAFLD. In our study, higher adherence to LCD was harmful to women's liver fat, while a DASH diet was protective against NAFLD. This study adds evidence that diet quality plays a key role in NAFLD development.

*

SATURATED FAT INTAKE FROM DAIRY SOURCES IS ASSOCIATED WITH REDUCED CARDIOVASCULAR RISK IN FRAMINGHAM OFFSPRING STUDY WOMEN

M. Yuan, R. T. Pickering, M. R. Singer, L. L. Moore

Nutrition and Metabolism Program

While saturated fat (SFA) intake has long been considered as an important risk factor for cardiovascular disease (CVD), some evidence in recent years has called these findings into question. The goal of this study was to determine whether higher (vs. lower) intakes of SFA from dairy and non-dairy sources were associated with risk of cardiovascular disease.

Data from 1991 adults, ages 30 and older, who were free of CVD at the time of baseline dietary assessment in the Framingham Offspring Study were included. Dairy and non-dairy SFA was assessed using 3-day diet records. Subjects were followed from exam 5 to exam 9 for CVD events. Cox proportional hazards models were used.

Subjects were classified into 3 categories of intake of dairy and non-dairy SFA. Women with moderate (vs. low) dairy SFA intakes had 56% (95% CI: 0.27-0.71) lower CVD risks, while women consuming high (vs. low) non-dairy SFA had 23% (CI: 0.51-1.14) lower risks. Neither dairy-based SFA nor non-dairy SFA intake was associated with CVD occurrence in men. Overall, subjects with higher intakes of dairy SFA combined with lower intakes of non-dairy SFA had the lowest risks of CVD (HR:0.73; 95% CI: 0.54-0.98).

Saturated fats derived from dairy sources were associated with a reduced risk of incident CVD in women.

Orofacial and Skeletal Biology Program

NOTE: Prize winners are noted in the following list of Abstracts by:

*1st Prize

The accompanying number indicates each abstract's poster board.

Participants
Yuhei Uda (*)

*

PROTECTIVE ROLE OF PARATHYROID HORMONE SIGNALING IN OSTEOCYTES AGAINST OXIDATIVE STRESS*

Y. Uda[1], C. Constantinou[1], T. Y. Huang[1]; J. W. Kin[1], C. Newell[1], P. Divieti Pajevic[1]

Orofacial and Skeletal Biology Program
[1] Department of Translational Dental Medicine, Henry M. Goldman School of Dental Medicine

Parathyroid hormone (PTH), a calcium regulator, is widely used to treat age-related osteoporosis, and its anabolic actions on bone are mediated by osteocytes, the most abundant cells in bone, via a G protein-coupled receptor (PPR). However, the role of PPR signaling in osteocytes during aging remains unclear. Therefore, this study aimed to uncover its role using an *in vivo* and *in vitro* model. Skeletal analysis of mice lacking PPR in osteocytes (Dmp1-PPR^{KO}) revealed age-induced osteopenia due to increased osteoclast number and activity and suppressed osteoblast activity compared to littermate controls. In these mice, at 13-month-old, the number of osteoprogenitors was reduced while marrow adiposity was increased compared to controls. Furthermore, in these mice, lack of PPR expression in osteocytes was associated with a significant increase in serum sclerostin, a potent inhibitor of bone formation, and an early onset of oxidative stress in osteocytes as demonstrated by immunostaining for 4-hydroxy-2-nonenals. *In vitro* studies using an osteocytic cell line (Ocy454-12H) showed that pretreatment with PTH significantly reduced hydrogen peroxide (H_2O_2)-induced cell death, measured by resazurin-based assays. Intracellular levels of reactive oxygen species, assessed by 2′,7′-dichlorofluorescin diacetate, were also significantly suppressed in the PTH-treated cells upon H_2O_2 exposure. Furthermore, PTH pretreatment significantly reduced p21/*Cdkn1a*, an activator of cellular senescence, and senescence-associated β-galactosidase expression in Ocy454-12H. These protective effects of PTH were not observed in cells, in which the PPR expression was ablated using CRISPR/Cas9 technique. Taken together these results demonstrate that PPR signaling protects osteocytes from oxidative stress and cellular senescence.

Department of Pathology & Laboratory Medicine

Participants
Thomas Bellio (38)

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DEFICIENCY OF BRAIN-DERIVED INSULIN-LIKE GROWTH FACTOR 2 (IGF2) PROMOTES SEIZURES AND EXACERBATES COGNITIVE DEFECTS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE (AD): EFFECTS OF PERINATAL CHOLINE SUPPLEMENTATION.

T. Bellio, S. Yee, T. Jayasinghe, V. Chinthareddy, M. Campion, H. Jamieson, S. Devaraj, J. Blusztajn, T. Mellott

Department of Pathology & Laboratory Medicine

High intake of the essential nutrient choline during early life increases brain expression of the growth factor IGF2 and protects Alzheimer's disease (AD) model mice from several features of AD-like pathology. The goals of this study are to understand if IGF2 mediates the beneficial effects of perinatal choline supplementation and to examine the effects of reducing brain-derived levels of IGF2 in AD model mice. We generated *Igf2fl/fl* wild-type and APPswe/PS1dE9 (APP.PS1) mice with a tamoxifen-inducible, forebrain-specific *Camk2a*-promoter-driven Cre recombinase (CAMK2a-ERT2). The pregnant and nursing mothers of these progeny consumed a control- or a choline-supplemented diet, and all offspring consumed a control diet after weaning. The forebrain-specific deletion of the *Igf2* gene was induced on postnatal day 60. At 6 months of age, there was a noticeable reduction in the levels of IGF2 protein in the hippocampus and cortex in mice with CAMK2a-CreERT2. There were no changes in the IGF2 levels within the liver of these mice suggesting that induction with tamoxifen specifically reduced forebrain-derived IGF2. As these tamoxifen-induced mice aged, we observed an increased mortality rate within APP.PS1 mice with CAMK2a-CreERT2 in both sexes, which may indicate increased vulnerability to AD in IGF2-deficient mice. Animal subjects were behaviorally tested at 6, 9 and 12 months of age. At 12-months of age, all male APP.PS1 mice exhibited a deficit during the acquisition training in the Barnes maze compared to all non-AD model mice, with APP.PS1/CAMK2a-CreERT2 mice being the most impaired during the training. Interestingly, 1-day probe testing in the Barnes maze revealed significantly impaired memory recall in APP.PS1/CAMK2a-CreERT2 mice compared to their IGF2-intact APP.PS1 counterparts. In addition, APP.PS1/ CAMK2a-CreERT2 mice were more prone to seizures during behavioral testing. These results show that long-term reduction of brain-derived IGF2 can be tolerated in non-AD mice but can further impair cognitive function in APP.PS1 mice. This suggests increased vulnerability to AD pathophysiology in these mice and supports the notion of a beneficial role of brain-derived IGF2 in later stages of AD progression.

Department of Pharmacology & Experimental Therapeutics

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
Jacob Beierle (*)
Jonique George (20)
Xuan (Anita) He (41***)
Caroline Murphy (6**)
Kristen Segars (24)
Chelsea Webber (6**)

*

A REDUCED COMPLEXITY CROSS BETWEEN BALB/c SUBSTRAINS IDENTIFIES *Zhx2* AS A CANDIDATE GENE UNDERLYING OXYCODONE METABOLITE BRAIN CONCENTRATION AND STATE-DEPENDENT LEARNING OF OPIOID REWARD*

J. Beierle, O. Averin, C. Byrant, A. Emili, M. Ferris, S. Goldstein, D. Moody, G. Peltz, C. Reilly, J. Scotellaro, K. Sena, A. Shah, E. Yao

Department of Pharmacology & Experimental Therapeutics

Understanding the pharmacokinetic profile of an opioid drug is vital to therapeutic success, and mutations in human PK genes can drastically alter therapeutic efficacy of opioids. We observed that at 30 min post-oxycodone administration BALB/cJ mice showed a higher whole brain concentration of oxycodone, and female specific increase in noroxycodone, and oxymorphone compared to BALB/cByJ. This observation could explain the sex-specific increase in oxycodone state-dependent conditioned place preference in BALB/cJ female mice. To potentially link behavioral differences with PK differences, we conducted quantitative trait locus (QTL) mapping of whole brain oxycodone and metabolite concentrations in a reduced complexity cross (RCC). Because BALB/cJ and BALB/cByJ substrains differ by ~8,500 SNPs/indels, large genetic loci identified in an F2 cross are offset by a dramatic reduction in potentially causal variants. QTL mapping in 133 BALB/cJ x BALB/cByJ F2 mice (68F, 65M) revealed a single QTL on chromosome 15 associated with brain oxymorphone concentration that explained 32% of the phenotypic variance in females. Oxymorphone is a full agonist at the mu opioid receptor, with 8x the potency of oxycodone, and likely contributes to oxycodone addictive properties. cis-eQTL analysis revealed genetically regulated expression of Zhx2, a transcriptional inhibitor known to harbor a private BALB/cJ retroviral insertion that dramatically reduces protein expression and leads to sex specific dysregulation of CYP450 genes within the liver. Whole brain mass spectroscopy proteomics in the parental strains corroborated these eQTL findings. We hypothesize that decreased Zhx2 expression leads to increased CYP450 expression, increased brain oxymorphone, and increased oxycodone-induced behaviors.

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TGF344-AD RATS DISPLAY DIFFERING LEVELS OF GABRA5 TRANSCRIPT IN SINGLE NEURONS OF THE CA1 DURING DISEASE PROGRESSION

J. George, D. Farb, V. Kumaresan, M. Ratner, S. Russek, A. Tipton

Department of Pharmacology & Experimental Therapeutics

In the United States, 5.5 million individuals suffer with Alzheimer's Disease (AD), characterized by a decline in cognition and the lethal disruption of all brain functions. The pharmacological administration via oral gavage of the cognitive enhancer a5IA, a negative modulator of the α5- containing γ-aminobutyric acid A receptor ($GABA_AR$), increases sharp wave ripple oscillations in wild-type rats but not in the transgenic AD (TgF344-AD) rat. We hypothesize that this loss of sharp wave ripple modulation reflects a molecular reorganization of trisynaptic hippocampal function. Towards this goal, we used a novel fluorescent *in situ* hybridization technique, RNAscope, to interrogate the spatial expression of *Gabra5* in dissected brains of TgF344-AD and littermate control wild-type rats (Ct), n=4, at two ages, 9mo and 16mo, to determine if there is a change in transcript abundance in the hippocampus. Our results show that in the CA1 of 16mo TgF344-AD rats, the expression of *Gabra5* across all neurons is decreased, and they display a decrease in the average number of *Gabra5* transcripts per cell. This decrease was not due to an overall decrease in CA1 neurons, indicating that the decrease in *Gabra5*$^+$ neurons in the aged TgF344-AD rats was due to a decreased *proportion* of *Gabra5*$^+$ neurons in the CA1 population, and suggestive of a homeostatic response to the beginnings of circuit dysfunction.

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IDENTIFICATION OF REGULATORY ELEMENTS ACTIVE IN SKELETAL TISSUES ASSOCIATED WITH CRANIOSYNOSTOSIS RISK GENES

X. He, A. Berenson, M. Bernard, C. Weber, J. F. Bass, and S. Fisher

Department of Pharmacology & Experimental Therapeutics

Craniosynostosis (CS), one of the most common craniofacial birth defects, is when one or more cranial sutures are prematurely replaced by bone, which reduces flexibility of the skull and restricts brain expansion. While a minority of cases are caused by single gene mutations, in the majority the genetic risk is more complex. A genome-wide association study (GWAS) for CS identified two risk loci, one downstream of *BMP2*, and one within the *BBS9* gene, adjacent to *BMPER*, encoding an extracellular modulator of BMP signaling. We hypothesized that distal regulatory elements for *BMPER* located within *BBS9* accounted for the CS risk and aimed to identify *BMPER* enhancers active in skeletal tissues. From a 1.3 Mbp region encompassing *BMPER* and a portion of *BBS9,* including the GWAS risk locus, we selected conserved noncoding sequences as candidates. Using zebrafish transgenesis, which provides temporal and tissue contexts of enhancer activities, we identified two enhancers. The -117*BMPER* is broadly active in early osteoblasts, whereas -707*BMPER* regulates expression in cranial cartilage and harbors a sequence variant linked to CS risk. We similarly examined the risk locus near *BMP2* and identified two enhancers; +402*BMP2* is active in bone, while +421*BMP2* active in cartilage and also contains a risk related variant. To further identify the potential transcription factor (TF) interactions with each enhancer, we screened them against a library of >1100 annotated human TFs via an enhanced yeast

one-hybrid (eY1H) assay. We are currently performing a targeted screening of the two enhancers containing CS risk-associated variants, to look for disease-related differences in TF bindings. In summary, we have found conserved enhancers for genes in the BMP pathway, and through the eY1H screen, identified signaling pathways that may regulate their activity. Both genes play conserved roles in skeletal development, and our analysis offers insights into their regulation across species. Both genes are also implicated in genetic risk for CS, and our identification of enhancers from the risk loci is a critical step in understanding the mechanisms underlying CS pathogenesis.

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CELL-CELL COMMUNICATION IN CENTRAL AND LIMBAL CORNEAL REGIONS IS DRIVEN BY CONDUCTOR CELLS

K. Segars, N. Azzari, C. Rich, V. Trinkaus-Randall

Department of Pharmacology & Experimental Therapeutics

When a cornea is injured, ATP release from damaged cells activates the purinergic receptor P2X7, which localizes to the wound edge within two hours of injury. This correlates with the presence of prominent calcium signaling events that have a high probability of communication between adjacent cells. Our goal is to determine if calcium signaling differs between the corneal epithelium and the corneal-limbal interface.

Live cell imaging was performed on both *ex vivo* globes from male C57Bl6 mice and cultured Human Corneal Limbal Epithelial (HCLE) cells using the Zeiss LSM 880 confocal microscope. Globes were pre-incubated with CellMask DeepRed and Fluo-4AM, and stabilized using a 3D printed holder. Images were collected after injury on the corneal epithelium and corneal-limbal regions. SiRNA knockdown was performed on HCLE cells prior to staining with SiR actin and Fluo-4AM, wounding and imaging. Analysis was performed using Matlab and ImageJ.

Calcium signaling was detected at the central cornea and at the corneal-limbal interface. In the limbal region, signaling occurs adjacent to nerves. In the central cornea, signaling occurs in basal cells adjacent to the wound in 9-week but not 27-week mice. Using machine learning we identified a subpopulation of HCLE cells that signal at a greater frequency and duration than others in the late wound response, and initiate cell-cell communication events. Knockdown of P2X7 reduces the number of conductor cells, and knockdown of Pannexin-1 causes the loss of a distinct conductor cell identity. Future directions will involve analyzing this conductor cell population in *ex vivo* globes.

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ORF57 FROM KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS IS PROTECTIVE AGAINST OXIDATIVE STRESS AND MAY BE A NOVEL BIOTHERAPEUTIC FOR NEURODEGENERATIVE DISEASE

C. J. Webber*, C. Murphy*, and B. Wolozin

Department of Pharmacology and Experimental Therapeutics
*These authors contributed equally to the work

In neurodegenerative disease, such as Alzheimer's disease (AD) and Amyotrophic lateral sclerosis (ALS), there is chronic activation of the integrated stress response (ISR). This pathway responds to cellular stress and protects the cell by halting global protein synthesis and promoting stress granule (SG) assembly. In disease, chronic activation leads to dysregulation of this pathway and protein aggregation. Pharmacological inhibitors of Protein Kinase R (PKR), an upstream regulator of the ISR, prevent the protein aggregation normally observed in AD. Unfortunately, these inhibitors are toxic *in vivo*. ORF57 from Kaposi's sarcoma-associated herpesvirus (KSHV) is an alternative approach to targeting the ISR and inhibiting PKR. ORF57 binds to PKR and prevents its activation during stress. We expressed ORF57 in human neuroblastoma cells (SH-SY5Y) and demonstrated that it is protective against oxidative stress.

ORF57 expressing cells exposed to stress had a significant reduction in phosphorylation of eIF2α compared to control cells (36.57 +/- 4.56% vs 67.98 +/- 22.97%, respectively $p<0.05$). This reduction is indicative of ORF57 successfully inhibiting the ISR. ORF57 expression increased protein translation in the SUnSET assay compared to control cells. ORF57 expression significantly decreased cleaved caspase 3 levels, an indicator of apoptosis, by more than 50% compared to control cells. ORF57 also significantly reduced G3BP1 and eif3η positive stress granules under stress. In order to determine if these benefits also translated to a disease model, we expressed ORF57 in SH-SY5Y cells with inducible TDP-43ΔNLS overexpression. TDP-43 aggregation is observed in 95% of sporadic ALS cases and in over 50% of AD cases. In these cells, ORF57 significantly decreased SG assembly and altered TDP43 aggregation. Using ORF57 to inhibit activation of the ISR is a novel approach to inhibiting the chronic activation of this pathway observed in neurodegenerative diseases.

Department of Physiology and Biophysics

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
Andrew Chang (2)
Emily Lewkowicz (*)

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EVOLUTION OF NEURONAL ACTIVITY AND SYSTEM ENTROPY IN C. ELEGANS UNDER ANESTHESIA

A. Chang, C. Connor, C. Gabel

Department of Physiology and Biophysics

Although volatile anesthetics are widely used in modern medicine, and are essential to the practice of surgery, the mechanisms by which they exert their powerful but reversible effects on nervous system is largely undetermined. The anesthetized state is characterized by a suite of behavioral and physiological responses, as well as by characteristic alterations in EEG patterns. However, these measures tell us little about the neuron or circuit-level physiological action of anesthetic agents. To probe the basis of volatile anesthetic action, we have utilized single-neuron calcium imaging in the C. elegans head ganglia to track pan-neuronal activity during emergence from anesthesia with isoflurane. Animals were continuously imaged at 2 volumes per second over 2 hours of emergence. Power spectral density analysis of individual activity traces obtained from anesthetized (n=10) and control exposure (n=8) animals demonstrated significant elevations in high frequency activity in anesthetized animals in the first three 12-minute epochs following withdrawal of isoflurane. Additional analysis was performed using both novel information theoretic metrics developed based on how entropy, or information content, is shared in the time-evolution of signals from paired neurons. These metrics allow us to consider how information flow is altered during the anesthetized state, and how this flow returns to baseline during emergence. Traditional entropy-derived metrics such as mutual information and transfer entropy did not strongly distinguish between anesthetized and non-anesthetized animals. However, novel metrics characterizing the shared entropic state of the system in alternative ways (termed state decoupling, severed predictability, and consistency) not only strongly served to differentiate the awake and anesthetized states (control vs. 4% and 8% isoflurane exposure), but additionally resolved over time during roughly two hours of emergence from 4% isoflurane exposure back to baseline levels (as compared to controls). The slow resolution of these metrics over hours contrasts strongly with the rapid resolution of high frequency activity immediately post-anesthesia, which suggests discrete phases of recovery from anesthesia and an emergence process during which distinct neurophysiological processes may resolve from a perturbed state to baseline at different rates.

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STRUCTURAL BASIS FOR THE ACTION OF HEPARAN SULFATE AND OTHER PERIODIC POLYANIONS AS UBIQUITOUS AMYLOID AGONISTS*

E. Lewkowicz, O. Gursky

Department of Physiology and Biophysics

Amyloidoses such as Alzheimer's, prion disease, inflammation-linked AA amyloidosis, etc. are incurable diseases wherein normally soluble proteins/peptides deposit as insoluble fibrils in various organs. Better mechanistic understanding of amyloid formation and stabilization will improve therapeutic targeting of amyloidoses. Besides proteins, ex-vivo amyloid deposits contain other components like heparan sulfate (HS). HS promotes amyloid formation by many unrelated proteins in vivo and in vitro, is an obligatory cofactor for tau fibril deposition, and is a therapeutic target in AA amyloidosis. Our goal is to provide the structural basis for understanding why HS and other polyanions act as amyloid agonists.

All known high-resolution structures of protein amyloid fibrils contain stacks of parallel in-register cross-β-sheets with arrays of identical residues ~4.7 Å apart. For charged residues, such arrays are energetically unfavorable unless compensated by opposite charges. We propose that HS, which is a periodic polyanion with ~5Å charge-charge separation, binds to basic residue arrays to nucleate and stabilize amyloid.

We tested this idea using flexible docking of a heparin tetrasaccharide to numerous high-resolution ex-vivo amyloid fibril structures from neurodegenerative and systemic amyloidoses. The results revealed that heparin's sulfates and carboxylates coordinate basic residue arrays in amyloid via charge-charge interactions. Our docking studies identified many potential heparin/HS binding sites that correspond to extra densities in high-resolution cryoEM maps. Ongoing molecular dynamics simulations using the ex-vivo human SAA fibril structure show charge-mediated heparin-fibril interactions that are consistent with experiments. This binding mode helps demonstrate how various polyanions promote nucleation and growth of amyloid fibrils.

Notes