4th Annual University of Colorado MSTP Retreat

Nighthorse Campbell Building

February 25th, 2022

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Retreat Schedule

Time	Session	Location
8:30-9AM	**Registration and Coffee**	Nighthorse Campbell Atrium
9-9:05AM	**Opening remarks and Welcome** Kelsey Kines	Shore Family Forum
9:05-9:30AM	**State of the program address** Cara Wilson, MD	Shore Family Forum
9:30-10:30AM	**Student oral abstract presentations** 9:30-9:45 Bruce Edward Kirkpatrick 9:45-10:00 Lily Nguyen 10:00-10:15 Annika Gustafson 10:15-10:30 Laurel Darragh	Shore Family Forum
10:30-10:45AM	**1 minute student lightning flash talks** Varuna Nangia Cecilia Levandowski Sarah Zych Jacqueline Turner Raquel Ortega Thomas Forman Frances Li Nkolika Egbukichi	Shore Family Forum
10:45-11AM	Break	
11:00-11:30AM	**Student poster session 1**	NHC Rm. 103
11:30-12:15PM	Lunch	
12:15 1:15PM	**Keynote address** Amy Clevenger, MD, PhD	Shore Family Forum
1:15-1:45PM	**Breakout session 1** (please choose your 1st session to attend) <u>Session A: Scientific Communication</u> Lauren Fishbein, MD, PhD Associate Professor of Medicine-Endocrinology/Metabolism <u>Session B: Scientific and Medical Distrust</u> Donald Nease, MD Professor of Family Medicine Montelle Tamez Deputy Director of Community Engagement at University of Colorado CTSI <u>Session C: Residency Panel</u> Devang Amin, MD, PhD - Internal Medicine - Cardiology	Session A: Shore Family Forum Session B: NHC Rm. 202 Session C: NHC Rm. 304/305

	Suguna Narayan, MD, PhD - Pathology Haley Simpson, MD, PhD Internal Medicine Hematology/Oncology	
1:50-2:20PM	**Breakout session 2** (please choose your 2nd session to attend) <u>Session A: Scientific Communication</u> Lauren Fishbein, MD, PhD Associate Professor of Medicine-Endocrinology/Metabolism <u>Session B: Scientific and Medical Distrust</u> Donald Nease, MD Professor of Family Medicine Montelle Tamez Deputy Director of Community Engagement at University of Colorado CTSI <u>Session C: Residency Panel</u> Devang Amin, MD, PhD - Internal Medicine - Cardiology Suguna Narayan, MD, PhD - Pathology Haley Simpson, MD, PhD Internal Medicine Hematology/Oncology	Session A: Shore Family Forum Session B: NHC Rm. 202 Session C: NHC Rm. 304/305
2:20-2:30PM	Break	
2:30-3:00PM	**Student poster session 2**	NHC Rm. 103
3:00-4:00PM	**Student oral abstract presentations** 3:00-3:15 Amita Kashyap 3:15-3:30 Meghan Kellett 3:30-3:45 Nathaniel (Nat) Skillin 3:45-4:00 Meagan Chriswell	Shore Family Forum
4:00-4:15PM	**Retreat wrap-up and awards** Retreat Planning Committee	Shore Family Forum
4:30PM	**Gather at Cedar Creek for happy hour**	Cedar Creek Pub

COVID Protocols

The guidelines below were developed in coordination with and with the full approval of both the university COVID committee/facilities and the SOM.

- **Please remain masked at all times unless you are actively eating or drinking.**
- **Open cans and cups are NOT allowed.** Any beverages served will have a lid or a cap. Please consider bringing your own water bottle.
- **Speakers may remove their mask when they are presenting. Poster presenters must remain masked.**
- During speaker sessions and breakout sessions, utilize all the space and 6ft distance as much possible.
- **Lunch has been approved to be eaten inside. Avoid eating in the Nighthorse Campbell Atrium. Please use the Shore Family Forum and Rms. 202, 304, and 305 to eat.** Please socially distance while eating and promptly re-masked when finished eating.
- **DO NOT attend if you are feeling any symptoms** (runny nose, cough, fever, body aches, sore throat, etc.)
- While not required, consider getting a COVID test prior to and after the retreat. You can schedule one at https://covidcheckcolorado.org/

Retreat Speakers

Dr. Amy Clevenger, MD, PhD Keynote Speaker

Amy Clevenger, M.D., Ph.D. is an Associate Professor of Pediatrics in the Section of Pediatric Critical Care Medicine at the University of Colorado Anschutz. She attended Stanford University and graduated with honors in Biological Sciences. She subsequently attended the Medical Scientist Training Program at the University of Colorado Health Sciences Center, obtaining her Ph.D. in Neuroscience. She completed her residency in Pediatrics at Washington University and St. Louis Children's Hospital and stayed an additional year there as one of three Pediatric Chief Residents. She then completed a Pediatric Critical Care Fellowship at the University of Pennsylvania and Children's Hospital of Philadelphia. She joined the Anschutz Medical Campus for her first faculty position. Her research seeks to better understand the cellular and molecular mechanisms of secondary brain injury in animal models of pediatric traumatic brain injury and ischemic stroke.

Dr. Cara Wilson, MD MSTP Director, Program Address

Cara Wilson, M.D. is a Professor of Medicine in the Division of Infectious Diseases at University of Colorado at Denver and holds a secondary appointment in the Department of Immunology and Microbiology. She is the director of the Medical Scientist Training Program at the University of Colorado. She received her medical degree from the University of Virginia School of Medicine and completed her residency training in Internal Medicine at Johns Hopkins Hospital. She subsequently completed Infectious Diseases fellowship training at Massachusetts General Hospital. Her laboratory studies the human immune response to HIV-1 infection and the factors that drive HIV-1 pathogenesis, particularly in intestinal mucosal tissue. Her most recent studies have focused on understanding the complex interactions between virus, gut commensal bacteria, and intestinal immune cells that contribute to mucosal and systemic inflammation during HIV-1 infection. She also extensive experience in designing and implementing HIV clinical trials through her involvement in the national AIDS Clinical Trials Group (ACTG), with an emphasis on studies of HIV-associated immune activation and immune-based vaccines and therapies.

Student Oral Presentations

Please vote for your favorite student oral presentation! Recognition will be given to the winners at the end of the retreat.
Link for voting: https://forms.gle/SNwPjUC1VpaFYfD37

Session 1:

1. Bruce E. Kirkpatrick

Intracellular Crowding by Bio-Orthogonal Hydrogelation Induces Reversible Molecular Stasis

Anseth Lab, Chemical & Biological Engineering, MSTP Class of 2018, GS2

2. Lily Nguyen

Combinatory epigenetic and PARPi therapy re-sensitizes resistant ovarian cancer cells to treatment and re-activates transposable elements at the transcript and epigenomic level

Bitler and Chuong Labs, Molecular, Cellular, & Development Biology, MSTP Class of 2017, GS3

3. Annika Gustafson

Elucidating the mechanism through which SIX1 regulates a myogenic progenitor-like state in rhabdomyosarcoma.

Ford Lab, Molecular Biology, MSTP Class of 2019, GS1

4. Laurel Darragh

The role of lymph nodes in priming an anti-tumor immune response both locally and distantly in head and neck cancer.

Karam Lab, Immunology, MSTP Class of 2017, GS3

Session 2:

1. Amita Kashyap

Determining the Fate of Pulmonary iILC2 Cells in Helminth Infection

Reinhardt Lab, Immunology, MSTP Class of 2019, GS1

2. Meghan Kellett

Investigating how nucleolar FAK drives growth and survival in thyroid cancer

Schweppe Lab, Cancer Biology, MSTP Class of 2016, GS4

3. Nathaniel (Nat) Skillin

Liquid Crystal Polymers for Muscle Tissue Engineering

Anseth and White Labs, Chemical & Biological Engineering, MSTP Class of 2018, GS2

4. Meagan Chriswell

Dual IgA/IgG family autoantibodies from individuals at-risk for rheumatoid arthritis identify an arthritogenic strain of Subdoligranulum

Kuhn Lab, Immunology, MSTP Class of 2016, GS4

Student Lightning Talks

Please vote for your favorite student oral presentation! Recognition will be given to the winners at the end of the retreat.
Link for voting: https://forms.gle/SNwPjUC1VpaFYfD37

Varuna Nangia
The role of non-canonical MAPK dynamics in non-genetic drug adaptation in melanoma
Spencer Lab, Chemistry & Biochemistry, MSTP Class of 2019, GS1

Cecilia Levandowski, PhD
Diabetes, drinking and dirt: a cautionary tale of disseminated nocardiosis
Hanna Lab, Chemistry & Biochemistry, MSTP Class of 2012, MS4

Raquel Ortega
Role of chromatin on alternative end-joining and PARPi resistance
Bilter and Arnoult Labs, Molecular, Cellular, & Development Biology, MSTP Class of 2019, GS1

Sarah Zych
Neuromodulators independently regulate striatal dopamine and GABA co-transmission
Ford Lab, Neuroscience, MSTP Class of 2015, GS5

Jacqueline Turner
Lipid signaling as a tolerogenic mechanism to modulate adaptive CD8 T cell immunity
Torres Lab, Pharmacology, MSTP Class of 2018, GS2

Thomas Forman
Investigating Srsf3-mediated alternative RNA splicing downstream of PDGFRalpha signaling in craniofacial development
Fauntauzzo Lab, Molecular Biology, MSTP Class of 2018, GS2

Frances Li
Defining MARCO-virus Interactions Important for Alphavirus Clearance from the Circulation
Morrison Lab, Microbiology, MSTP Class of 2017, GS3

Nkolika Egbukichi
FAK and ECM Stiffness
Schweppe Lab, Cancer biology, MSTP Class of 2018, GS1

Poster Sessions

Session A 11:00-11:30

1. **Anagha Inguva**
The Role of BCL2 Mediated Calcium Signaling in Leukemia Stem Cell Metabolism and Function
Cancer Biology, GS4

2. **Brenda Seymour**
Indole promotes collagen-induced arthritis through enhanced Th17 immunity
Immunology, GS2

3. **Carley Miller**
A role for ventral pallidum glutamate neurons in the effects of stress on heroin sensitization
Pharmacology, GS1

4. **Connor Hughes**
Investigating the role of Eya3 in the regulation of NF-kB signaling and tumor progression in TNBC
Pharmacology, GS4

5. **Emily King**
Recruitment of macrophages to the airspace and pulmonary interstitium in inflammation
Immunology, GS2

6. **Jordan Hickman**
Demystifying the effects of electrical brain stimulation on neural activity with multineuropixel recordings in mice
Neuroscience, GS1

7. **Juan Santiago**
Color Representation in the Primary Visual Cortex
Neuroscience, GS2

8. **Kelsey Kines**
The role of Semaphorin 7A in aged and parous breast tissue and its role in promoting breast cancer metastases.
Cancer Biology, GS2

9. **Michael Nash**
Maternal western diet programs inflammatory trained immunity in fetal and juvenile non-human primates through hematopoietic stem cells and macrophages
Integrated Physiology, GS4

10. **Cecilia Levandowski, PhD**
Diabetes, drinking and dirt: a cautionary tale of disseminated nocardiosis
Chemistry & Biochemistry, MS4

11. **Uma Kantheti**
Role of PD-L1 Signaling in Dermal Dendritic Cell Migration
Immunology, GS1

12. **William Sheeran**
Neuronal ensembles underlying monogamous behavior in nucleus accumbens.
Molecular, Cellular, and Developmental Biology, GS2

Session B 2:30-3:00

1. **Amelia Burch**
CD38 Activation of the TRPM2 Ion Channel Contributues to Post-Stroke Hippocampal Dysfunction
Pharmacology, GS3

2. **Austin Jolly**
The Chromatin Remodeling Protein Brg1 Regulates Adventitial Progenitor Cell Myofibroblast Differentiation and Pathological Vascular Remodeling and Fibrosis
Pharmacology, GS4

3. **Brian Lloyd**
Neurexin-3 controls excitatory synaptic nano-organization
Pharmacology, GS3

4. **Chris Alderman**
Determining the mechanism of action of a small molecule compound targeting eIF3
Molecular Biology, GS3

5. **Dylan Calame**
Cerebellar Associative Learning Underlies Skilled Reach Adaptation
Neuroscience, GS5

6. **Hei-Yong (Grant) Lo**
What RNAs localize to the centrosome and how do they get there?
Molecular Biology, GS3

7. **Joseph (Joe) Hsieh**
Mechanisms of PAX3-FOXO1 Cofactors Chromatin Recruitment in Fusion-Positive Rhabdomyosarcoma
Cancer Biology, GS3

8. **Keith Dodd**
Changes in functional connectivity of the striatum correlated with relative weight-gain risk of antipsychotics
Bioengineering Medical Imaging, GS1

9. **Kitz (David Kitzenberg)**
Adenosine awakens metabolism to enhance growth-independent killing of tolerant and persister bacteria across multiple classes of antibiotics
Molecular Biology, GS4

10. **Mostafa El-Kalliny**
Interrogating the role of inhibitory interneurons of the nucleus accumbens in social attachment
Molecular, Cellular, & Development Biology, GS1

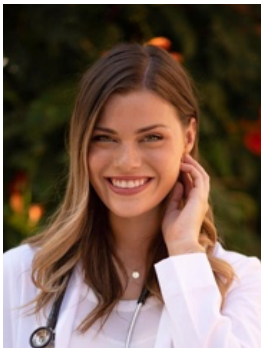
11. **Taylor Yamauchi**
Investigating circuits for target acquisition
Neuroscience, GS4

12. **Shanawaj (Roy) Khair**
Does a single ethanol exposure prior to burn injury in mice worsen pulmonary inflammation as much as episodic exposure of ethanol?
Molecular Biology, GS3

13. **Faye Camp**

Acknowledgments

Thank you to the 2022 Retreat Planning Committee!



Kelsey Kines

Sarah Zych

Meagan Chriswell

Roy Khair

Bruce Kirkpatrick

Carley Miller

Selin Ekici

Thank you to AMBOSS for providing the 12-minute talk award!

AMBOSS

Student Abstracts

Alphabetical by student first name

Amelia Burch

CD38 Activation of the TRPM2 Ion Channel Contributes to Post-Stroke Hippocampal Dysfunction

GS3, Pharmacology

Amelia Burch, Danae Mitchell, James Orfila, Andra Dingman, Nidia Quillinan, Paco Herson

Emerging clinical data reveals post-stroke cognitive impairment (PSCI) as one of the leading causes of disability in the country. Recently, our lab has identified the TRPM2 ion channel as a potential molecular target for the reversal of cognitive symptoms following global cerebral ischemia. Whether TRPM2 channel modulation is of benefit in the context of focal ischemia and PSCI remains an open question. To address this, we perform a 60-minute transient middle cerebral artery occlusion (MCAO) in adult male mice as a preclinical model for stroke and assess synaptic plasticity in combination with neurobehavior with a focus on the hippocampus, a region known to contribute to PSCI. To measure hippocampal synaptic plasticity, we employ field electrophysiology and assess long-term potentiation (LTP) at delayed (30 day) timepoints following MCAO. We find that LTP is reduced following MCAO (n=3, 15.0±4.0%) compared to sham (n=6, 47.2±9.2%; $p<0.05$). However, i.v. treatment with tat-M2NX (20mg) on day 29, a specific inhibitor of TRPM2, fully restores LTP by day 30 (n=6, 65.3±8.1%). We next investigate potential mechanisms of stroke-induced TRPM2 activation with a focus on CD38, an ectoenzyme and potential source of the TRPM2 ligand, ADP-ribose (ADPr). Using immunohistochemical (IHC) staining, we show CD38 is localized to activated astrocytes in the hippocampus following MCAO. We also find impaired hippocampal LTP on MCAO acute brain slices is restored to sham levels following bath application of apigenin (vehicle n=7, 23.9±7% vs. 10μM apigenin n=7, 65.5±2.5%; $p<0.05$), and 78c (vehicle n=6, 4.0±8.5% vs. 100nM 78c n=6, 78.3±15.8%; $p<0.05$), phenocopying TRPM2 inhibition. Taken together, these data identify the TRPM2 channel as a contributor to stroke-induced hippocampal dysfunction, with the astrocyte-localized ectoenzyme, CD38, as an ADPr source and potential upstream regulator of TRPM2, uncovering a novel neuroglial pathway underlying PSCI.

Amita Kashyap

Determining the Fate of Pulmonary iILC2 Cells in Helminth Infection

GS1, Immunology

Innate Lymphoid Cells (ILC) are innate immune counterparts to T-cells, with Type 2 ILC (ILC2) counterparts to Th2 cells and key early responders in helminth infection, capable of controlling helminth infection even in Rag-deficient mice. Until recently, ILC2 had been considered a homogenous population, but recent work delineated two sub-populations: "inflammatory" iILC2 which appear transiently in the lungs during the response to helminth infection, and "natural" nILC2, present in peripheral tissues regardless of infection status. While nILC2 undergo population expansion following helminth infection, the ultimate fate of the transient iILC2 population remains

undetermined. While there is evidence to suggest that iILC2 are capable of conversion to nILC2-like phenotype, dogma in the field maintains that nILC2 populations are maintained solely by self-renewal. Our preliminary data from congenic transfer experiments and single-cell sequencing, however, suggests that iILC2 convert to an nILC2 phenotype in vivo. We therefore hypothesize that iILC2 convert to an nILC2 phenotype and make a long-term contribution to the tissue-resident nILC2 population following helminth infection in vivo.

Anagha Inguva
The Role of BCL2 Mediated Calcium Signaling in Leukemia Stem Cell Metabolism and Function
GS4, Cancer Biology
Anagha Inguva*, Shanshan Pei, Hunter Tolison, Maria L. Amaya, Anna Krug, Mohd Minhajuddin, Brett M. Stevens, Daniel A. Pollyea, Craig T. Jordan
Leukemia stem cells (LSCs) initiate AML and are ineffectively eradicated by chemotherapy, thus causing relapse. LSCs have a unique dependence on oxidative phosphorylation (OXPHOS) for energy production, which can be perturbed by BCL2 inhibition. Recently FDA approved venetoclax (BCL2 inhibitor) for older patients with AML shows effective killing of LSCs in patients via metabolic perturbation. Approximately ~50% of patients are refractory or relapse on therapy, therefore understanding the relationship between BCL2 and LSC biology will lead to novel therapies for non-responders. The mechanism between BCL2, a canonical anti-apoptotic protein and perturbation of metabolism including the OXPHOS pathway remains unclear. BCL2 has a well established non-canonical role in calcium signaling by binding to and regulating multiple calcium channels including: SERCA, IP3R, and VDAC. The role of BCL2 in calcium regulation could link BCL2 and OXPHOS as the three rate limiting steps of the TCA cycle are calcium-dependent reactions. Further, maintaining ideal mitochondrial calcium concentration is crucial as too little or too much mitochondrial calcium leads to TCA cycle shutdown and decreased OXPHOS activity. We therefore sought to investigate whether BCL2 can affect OXPHOS via regulation of calcium in LSCs. Using metabolic assays, proximity binding studies, mitochondrial calcium measurements and xenograft experiments we were able to show the following results in primary human AML patient LSCs: 1) BCL2 and its binding partners modulate mitochondrial calcium levels, metabolism and function of primary human AML stem cells 2) Venetoclax resistant LSCs have unique calcium biology properties that can be targeted. Future work to complete our studies includes testing novel therapeutic strategies that target calcium biology to overcome venetoclax resistance and developing a predictive algorithm to predict sensitivity to venetoclax based regimens in AML patients in the up-front setting.

Anand (Andy) Tekriwal
Context modulates basal ganglia output in patients with Parkinson's disease
Anand Tekriwal*, Gidon Felsen, Steven Ojemann, Aviva Abosch, John A. Thompson
Introduction: Patients with Parkinson's disease (PD) report varied motor symptom severity based on their environment. For example, the presence of external patterns such as a rhythmic tone can attenuate bradykinetic impairments. Decades of anecdotal

reports and behavioral studies describe this and related phenomena, yet the neural mechanisms for this context-dependent attenuation remain unknown.
Objectives: Here, we investigate whether context-dependent symptom attenuation is reflected in single-unit activity recorded in the operating room from the substantia nigra pars reticulata (SNr) of PD patients undergoing DBS. The SNr is known to influence motor planning and execution in animal models but its role in humans remains understudied.
Methods: Following IRB study approval, informed consent was obtained from 9 subjects (4 male) undergoing STN DBS for treatment of PD. We recorded SNr activity while subjects performed cued directional movements in response to auditory stimuli under interleaved "patterned" and "unpatterned" contexts. SNr localization was independently confirmed with expert intraoperative assessment as well as post-hoc imaging-based reconstructions.
Results: As hypothesized, we found that motor performance was improved in the patterned context, reflected in increased reaction speed and accuracy compared to the unpatterned context. These behavioral differences were associated with enhanced responsiveness of SNr neurons (n = 33 well-isolated units) i.e., larger changes in activity from baseline in the patterned context. Unsupervised clustering analysis revealed two distinct subtypes of SNr neurons: one exhibited context-dependent enhanced responsiveness exclusively during movement preparation, whereas the other showed enhanced responsiveness during portions of the task associated with both motor and non-motor processes.
Conclusions: Our findings indicate the SNr participates in motor planning and execution, as well as warrants greater attention in the study of human sensorimotor integration and as a target for neuromodulatory therapies.

Annika Gustafson
Elucidating the mechanism through which SIX1 regulates a myogenic progenitor-like state in rhabdomyosarcoma.
GS1, Molecular Biology
Gustafson, A.L., Hsu, J.Y., Artinger, K.B., and Ford, H.L.
Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma and is characterized by molecular and histologic features resembling undifferentiated skeletal muscle. Patients with metastatic or recurrent RMS have poor rates of overall survival and traditional chemotherapy regimens have a high degree of morbidity. RMS is molecularly categorized as either fusion-positive (FP-RMS), which is driven by a PAX3/7-FOXO1 chromosomal translocation, or fusion-negative (FN-RMS), which is driven by a variety of mutations including p53 loss or RAS activation. Both subtypes of RMS express high levels of the developmental transcription factor SIX1, which is known to be a critical regulator of myogenic differentiation during embryogenesis. SIX1 knockdown (KD) in normal muscle progenitor cells leads to a decrease in expression of myogenic regulatory factors (MRFs) and halts differentiation. Intriguingly, our recent published work demonstrates that when SIX1 is knocked-down in FN-RMS, MRF expression increases, cell elongation and multi-nucleation occur, and MyHC expression increases, all indicative of terminal muscle differentiation. RNA-sequencing reveals that SIX1 KD in RMS globally alters gene expression to mimic that of terminally

differentiated muscle. Early experiments investigating SIX1 and its' EYA coactivators indicate that SIX1 levels increase at the initiation of skeletal muscle differentiation and then decline until myotubes are formed. We hypothesize that, FN-RMS is stalled in an undifferentiated state that mirrors the initiation of normal muscle development, where SIX1 is high, and that perturbation of SIX1 transcriptional complexes results in restoration of a normal developmental state. Understanding the dynamic alterations in DNA binding of SIX1 and its co-factors during myogenesis, and how that relates to FN-RMS, may enable the discovery of novel targets whose inhibition could restore normal developmental processes in this deadly pediatric disease.

Austin Jolly

The Chromatin Remodeling Protein Brg1 Regulates Adventitial Progenitor Cell Myofibroblast Differentiation and Pathological Vascular Remodeling and Fibrosis

GS4, Pharmacology
Austin Jolly*, Sizhao Lu, Allison Dubner, Marie Mutryn, Raphael Nemenoff, Mary Weiser-Evans

Cardiovascular diseases lead to long-term stiffening of arteries and small vessels. The vascular wall contains many cell populations that coordinate to maintain vessel homeostasis and induce vascular remodeling in disease states. We identified a population of progenitor cells that reside in the adventitial layer (AdvSca1-SM cells) that drive vascular fibrosis after acute vascular injury. AdvSca1-SM cells can differentiate into myofibroblasts and secrete pro-remodeling factors including extracellular matrix proteins and pro-inflammatory cytokines. While the response of AdvSca1-SM cells to vascular injury has been studied, the epigenetic changes that influence their differentiation towards myofibroblasts is poorly understood. We observed Brg1, a chromatin remodeling ATPase, is upregulated in AdvSca1-SM cells in response to vascular injury and may be a key factor in regulating gene expression to influence AdvSca1-SM differentiation. We hypothesize that Brg1 activates gene sets associated with remodeling and fibrosis and ultimately drives AdvSca1-SM differentiation towards pathologic myofibroblasts.

Results: Arteries from mice subject to carotid ligation exhibit decreased perivascular collagen deposition, smaller neointima, decreased numbers of alpha-actin positive AdvSca1-SM cells, and fewer infiltrating macrophages when treated with the pharmacological Brg1 bromodomain inhibitor PFI-3 as compared to mice that received control. In a cell culture system, TGF-□ induces AdvSca1-SM cells to express myofibroblast-specific genes, and demonstrate enhanced contractile function. PFI-3 blocks TGF-□ induced myofibroblast gene expression and dampens contractile function. Gene Ontology analysis reveals the top 100 TGF-□ inducible genes blunted by PFI-3 are related to fibrosis and extracellular matrix signaling. These results suggest Brg1 is a major regulator of vascular fibrosis and may be a targetable protein to help people who suffer from disease-related vascular stiffening.

Brenda Seymour
Indole promotes collagen-induced arthritis through enhanced Th17 immunity
GS2, Immunology
Brenda Seymour*, Brandon Trent, Sabrina Fechtner, Brendan Allen, Jimmy Tangchittsumran, and Kristine Kuhn
Altered tryptophan catabolism by the intestinal microbiota has been observed in autoimmunity, but the mechanisms by which tryptophan catabolites alter immune cell function are unclear. We found that mice fed a tryptophan-deficient (TD) diet are protected from collagen-induced arthritis (CIA) by alteration of the Th17:Treg ratio, and that levels of the bacterial-derived tryptophan metabolite indole correlate with CIA severity. Thus, we hypothesized that indole promotes CIA by skewing intestinal Th17 cell differentiation. To assess our hypothesis, mice were fed a TD diet starting at day -1. Indole (10mM) or vehicle was added back by oral gavage every other day. CIA was induced by immunization with bovine type II collagen in complete Freund's adjuvant at days 0 and 21. We found that indole-gavaged mice had a significant increase in disease severity and an expansion of Th17 cells at day 35 compared to vehicle-gavaged mice. Next, we evaluated if intestinal antigen presenting cells (APCs) would skew towards Th17 differentiation during CIA. We observed an expansion of IL-6, IL-23p19, and TNF-producing CD11c+MHCII+CD11bhi cells in the mesenteric lymph nodes of CIA mice at day 7, suggesting that cytokines important for Th17 cell function are upregulated early in CIA. To test whether indole promotes cytokine production in APCs, bone marrow derived dendritic cells were stimulated with indole +/- LPS for 24hr. Stimulation with indole + LPS resulted in significantly higher levels of IL-6, IL-23, and TNF compared to LPS alone. In conclusion, our initial data suggests that indole promotes CIA through enhanced Th17 cell differentiation.

Brian Lloyd
Neurexin-3 controls excitatory synaptic nano-organization
GS3, Pharmacology
Brian Lloyd*, Becca Roth, Jason Aoto
Synaptic adhesion molecules are a diverse class of proteins that participate in synaptic formation, maintenance, and function which are critical for efficient synaptic transmission and plasticity. Recent evidence suggests that synaptic adhesion molecules allow for effective synaptic transmission via clustering and alignment of presynaptic active zones, where neurotransmitter is released, and postsynaptic receptors. Neurexins (Nrxns) are a class of essential, disease relevant presynaptic adhesion molecules which have been proposed to modulate synaptic nano-organization due to their known roles in regulating pre and postsynaptic structure via intracellular and transsynaptic signaling respectively. Nrxns are encoded by three evolutionarily conserved Nrxn genes (Nrxn1, 2, and 3) which were initially proposed to be redundant at all synapses; however, it is becoming increasingly apparent that individual Nrxns govern distinct aspects of synapse function. For example, Nrxn3 has been shown to control AMPA receptor strength but how Nrxn3 dependent nano-organization may contribute to this effect remain unknown. Here, I am investigating the role of Nrxn3 in synapse nano-organization using our Nrxn3 conditional knockout mouse and double helix 3D STORM to examine the clustering and alignment of proteins critical for synaptic transmission and have shown that Nrxn3

is required for the organization and alignment of excitatory synapses. These data represent, to the best of our knowledge, the first evidence that neurexins, and more widely synaptic adhesion molecules, are required for the nano-organization of excitatory synapses and provides further insight into the mechanism by which Nrxn3 controls AMPA receptor strength. Future studies will focus on defining the endogenous localization of Nrxn3 at excitatory synapses to investigate if Nrxn3 is enriched in areas where its ligands are known to localize.

Brigit High
Species Differences in the Expression of P2X Receptors on Gustatory Nerves
GS4, Neuroscience
Brigit High*, Thomas E. Finger
The trimeric ionotropic purinergic receptors P2X2 and P2X3 are essential for the transmission of taste information from taste buds to gustatory nerves. In mice, gustatory nerve fibers express both P2X2 and P2X3, so many receptors are likely P2X2/P2X3 heteromers. P2X3 is also expressed homomerically in nerve fibers innervating the large airways which mediate cough triggered by ATP release from local tissues. Broad P2X3 antagonists have been used successfully to treat chronic cough in humans but produce dysgeusia likely due to off-target effects on P2X receptors in taste nerves. One avenue of thought is that antagonists specific for P2X3 homomers might still reduce objective cough frequency in chronic cough patients while avoiding off-target dysgeusic effects. Since P2X subunit composition may differ across species, we used immunohistochemistry to investigate taste bud innervation in humans (31 total samples 12 laryngeal, 19 fungiform) and in Rhesus monkey (5 monkeys, both laryngeal and fungiform tissues) to test whether the taste nerves in these species express both P2X2 and P2X3 as in mice. Antibodies to P2X2 and P2X3 were validated against hP2X2-expressing HEK cells and human gastrointestinal tissue containing P2X2-expressing neurons in the submucosal plexus. In fungiform taste bud samples from humans and monkeys, P2X3+ fibers extensively innervate taste buds as in mice. However, all Rhesus samples and most human samples lacked P2X2+ innervation. Of the 31 human subjects, only four (1 laryngeal, 3 fungiform) showed expression of P2X2 in nerve fibers innervating taste buds. These findings suggest that for most humans, taste buds are innervated by nerve fibers expressing only P2X3 homomeric receptors and not P2X2/P2X3 heteromers

Bruce Edward Kirkpatrick
Intracellular Crowding by Bio-Orthogonal Hydrogelation Induces Reversible Molecular Stasis
GS2, Chemical & Biological Engineering
Bruce E. Kirkpatrick*, Laura J. Macdougall, Timothy E. Hoffman, Benjamin D. Fairbanks, Sabrina L. Spencer, Christopher N. Bowman, Kristi S. Anseth
Intracellular biochemical equilibria exhibit substantial dependence on microenvironmental conditions. In particular, macromolecular crowding is an essential participant in the determination of diffusion and association rates in vivo. However, specific contributions are difficult to parse in the setting of exquisitely complex biochemical networks, especially as cells regulate their internal states in response to a

variety of intrinsic and extrinsic signals across length scales ranging from small molecules to protein assemblies on the order of MDa. In this work, we disrupt homeostatic biological functions by assembling synthetic hydrogels in the cytosol of living cells, which increases the excluded volume within the intracellular compartment. Hydrogelation following transfection with PEG macromer precursors is mediated by bio-orthogonal chemistries including azide-alkyne cycloaddition and thiol-ene photocrosslinking and results in a phenotype we term 'biostasis,' with global decreases in DNA transcription, protein synthesis, and cellular movement and proliferation. To quantify the physical effect of intracellular network formation, we utilize fluorescence correlation spectroscopy to measure the movement of fluorophores within these hydrogelated cells and identify significant increases in the effective viscosity of internal contents, suggesting a diffusion-based mechanism for inducing quiescence in cells via supraoptimal crowding. Moreover, these effects can be reversed by incorporating photodegradable chemistries into the hydrogel precursors, allowing for on-demand and spatiotemporal deactivation of the biostasis effect. This approach presents new opportunities for asserting control over cell cycle dynamics without specifically targeting biochemical pathways, as well as examining the relationship between biological activity and physical properties.

Carley Miller
A role for ventral pallidum glutamate neurons in the effects of stress on heroin sensitizatio
GS1, Pharmacology
Carley N Miller*, Nicholas M Fayette, Brandi Weidmeyer, & Jasper A Heinsbroek
Stress is an important risk factor for the development of opioid use disorder (OUD) and stressful life events often trigger relapse to OUD. Similarly, animal models show that acute stress modulates the acquisition and reinstatement of drug self-administration and alters the sensitized locomotor responses to drugs of misuse. Despite these behavioral correlates, our understanding of brain regions mediating the impact of stress on OUD is incomplete. One brain region of interest is the ventral pallidum (VP), a major downstream target of the mesolimbic reward system and a recipient of glutamatergic innervation from stress reactive brain regions. In particular, glutamatergic neurons of the VP have been linked to aversive behaviors (i.e. stress). Thus, our study sought to examine the role of VP glutamate neurons during exposure to acute stress and the subsequent development of locomotor sensitization to heroin using Vglut2-IRES-Cre mice. Mice were exposed to acute (1h) restraint stress 24h prior to undergoing heroin sensitization. During these procedures, VP glutamate neuronal activity was monitored in vivo with a miniscope system for imaging calcium activity in the VP using implanted GRIN lenses. These ongoing studies aim to better characterize the involvement of VP glutamate neurons in acute stress and behavioral sensitization to heroin.

Cecilia Levandowski, PhD
Diabetes, Drinking, and Dirt: A cautionary tale of disseminated nocardiosis.
MS4, Chemistry & Biochemistry
*Cecilia Blair Levandowski, PhD, Anna E. Buehler, MD, Juan N. Lessing, MD FACP, Reem M Hanna, MD

Case Information: A 72-year-old male soil excavator with controlled type-2 diabetes and alcohol use disorder presented with two months of progressive dyspnea. Evaluation revealed acute hypoxic respiratory failure with multifocal pneumonia and sodium of 119 mmol/L determined secondary to syndrome of inappropriate anti-diuretic hormone (SIADH). He was discharged after treatment for pneumonia but returned with worsening symptoms. During hospitalization he developed seizures, which led to imaging that revealed numerous brain micro-abscesses, cavitary lung nodules, adrenal gland abscess, and later chorioretinitis. Biopsy and cultures diagnosed disseminated Nocardia beijingensis. Despite 10 weeks of appropriate antibiotics (including imipenem, minocycline, ceftriaxone, trimethoprim-sulfamethoxazole, linezolid), his condition deteriorated, and family opted to transition to hospice.
Discussion: Nocardiosis is a disease caused by the gram-positive bacterium Nocardia, found in soil. While typically considered in the differential for the immunocompromised, up to one-third of patients are non-immunocompromised. As in this case, it is important to note that many common conditions, such as diabetes, heavy alcohol use, and advanced age, lead to a sufficient relative-immunocompromise that should prompt inclusion of nocardiosis in the differential. In-depth history-taking often reveals risk factors including working near soil. Pulmonary involvement is most common (71% of cases) and symptoms are nonspecific cough, dyspnea, and sputum production. Dissemination is reported in 11% of cases primarily involving the brain with neurologic symptoms. SIADH has diverse etiologies, and therefore often discounted. As in this case, SIADH has been reported as a presenting symptom in numerous case reports of both pulmonary and disseminated nocardiosis: Persistent SIADH in the context of non-resolving pneumonia should prompt consideration of nocardiosis. Early diagnosis and initiation of treatment is necessary to improve outcomes.

Chris Alderman
Determining the mechanism of action of a small molecule compound targeting eIF3
GS2, Molecular Biology
Chris Alderman, Rui Zhao
eIF3e, the eukaryotic initiation factor 3e, is a component of the 13-member elongation factor 3 complex. This complex plays an essential role in cap-dependent translation initiation of mRNA's. eIF3e has also been found to be a tumor suppressor and oncogene - emphasizing the role of combinatorics in cancer. Cancers it is involved in include high-grade gliomas, breast cancer, colon cancer, oral cancer, and NSCLC. We have discovered a small molecule with anticancer properties targeting eIF3e. These anticancer properties have been shown in cells as well as in a mouse metastasis model. My focus is determining the biochemical, biophysical, and structural mechanisms this small molecule uses to target eIF3e. In order to do this, we first needed to purify the eIF3e subunit in the eIF3a/c/e subcomplex to increase its in-vitro stability. Once we obtained a stable, purified complex, we began biochemical assays including Cellular Extract Thermal Shift Assays, Microscale Thermophoresis, and Isothermal Shift Assays to confirm a direct interaction between the small molecule and eIF3e. Next, to look at how it affects translation, we used co-immunoprecipitation of the eIF3 complex from treated or untreated cells to show our small molecule changes the eIF3 complex

component stoichiometry, and luciferase assays to determine the compound's effect on different 5' UTRs.

Connor Hughes
Investigating the role of Eya3 in the regulation of NF-kB signaling and tumor progression in TNBC
GS4, Pharmacology
Hughes, C.J.*, Rosenbaum, S., Vartuli, R.L., Fields, K., Zhou, H., Gustafson, A., Kong, D., Slansky, J., Zhao, R., and Ford, H.L.
Triple Negative Breast Cancer (TNBC), characterized by low/absent expression of the estrogen receptor and the HER2 growth factor receptor, has high rates of metastasis and an overall poor prognosis largely due to a lack of targeted therapeutic options. The Eyes absent (Eya) family of proteins are transcriptional cofactors with both intrinsic tyrosine phosphatase and associated serine/threonine phosphatase activities. In addition to developmental functions, Eyas have been shown to play a role in promoting many hallmarks of cancer. Published work from our laboratory demonstrated that Eya3 is capable of upregulating expression of PD-L1 in TNBC tumors thereby suppressing anti-tumor CD8+ T-cell responses, and preliminary data suggests that Eya3 may also regulate the expression of various cytokines in this context. In tumor cells, innate immune signaling pathways, such as NF-kB, have been shown to promote tumor progression, immune evasion, and metastasis in many distinct tumor types. We hypothesize that, in addition to regulating CD8+ T-cell responses, Eya3 regulates innate immune signaling in TNBC cells to facilitate enhanced primary tumor growth and metastasis. Preliminary findings suggest that Eya3 greatly enhances tumor progression and metastasis in TNBC and is a strong regulator of NF-kB signaling in this context. Additional preliminary data suggests that primary tumor cell-autonomous Eya3 expression may also be capable of regulating immune components of the pre-metastatic niche. Further exploration is warranted to determine the mechanism through which Eya3 regulates these phenotypes, whether Eya3-mediated NF-kB activation is a major contributor to the pro-metastatic phenotype of Eya3, and whether Eya3 could serve as a therapeutic target for preventing or treating disseminated TNBC.

Dylan Calame
Cerebellar Associative Learning Underlies Skilled Reach Adaptation
GS5, Neuroscience
Dylan J. Calame*, Matthew I. Becker, Abigail L. Person
Cerebellar output has been shown to enhance movement precision by scaling the decelerative phase of reaching movements in mice. We hypothesized that during reach, initial kinematics cue late-phase adjustments through cerebellar associative learning. We identify a population-level response in mouse PCs that scales inversely with reach velocity, suggesting a candidate mechanism for anticipatory control to target limb endpoint. We next interrogate how such a response is generated by combining high-density neural recordings with closed-loop optogenetic stimulation of cerebellar mossy fiber afferents originating in the pontine nuclei during reach, using perturbation schedules reminiscent of classic adaptation paradigms. We found that reach kinematics and PC electrophysiology adapt to position-locked mossy fiber perturbations and exhibit

aftereffects when stimulation is removed. Surprisingly, we observed partial adaptation to position-randomized stimulation schedules but no opposing aftereffect. A model that recapitulated these findings provided novel insight into how the cerebellum deciphers cause-and-effect relationships to adapt.

Emily King
Recruitment of macrophages to the airspace and pulmonary interstitium in inflammation
GS2, Immunology
Emily M. King*, Thienthanh Trinh, Jazalle McClendon, Peter K. Moore, Alexandra L. McCubbrey, Peter M. Henson, William J. Janssen
During inflammation circulating monocytes are recruited into the lung where they differentiate into macrophages. Recruited macrophages can contribute to both the airspace macrophage (AM) and the interstitial macrophage (IM) compartments. Two IM subsets have been identified and are distinguished by expression of the scavenger receptor CD163, however the turnover and fate of these subsets is not well understood. This study aims to establish the pattern of monocyte recruitment to the IM compartment during homeostasis and inflammation. Mice were treated with intraperitoneal injection of 5-ethynyl-2-deoxyuridine (EdU) to label bone marrow precursor cells followed by intra-tracheal (i.t.) instillation of E. coli LPS to initiate inflammation. Lungs were harvested after LPS treatment, and EdU incorporation and Ki67 labeling of macrophages was analyzed. Under homeostatic conditions, in the IM compartment approximately 10% of CD163- IMs were labeled with the proliferation marker Ki67 at all time points but 20-30% were labeled with EdU suggesting ongoing recruitment of EdU-labeled monocytes from the circulation. Only 10% of CD163+ IMs were labeled with EdU or Ki67 indicating minimal contribution from monocytes and minimal proliferation. One day after i.t. LPS up to 70% of the CD163- IMs were labeled with EdU and up to 45% of CD163+ IMs, reflecting monocyte contribution to both IM subsets. This proportion remained elevated at 40% in the CD163- population six days after i.t. LPS treatment, however only 10% of the CD163+ IMs were EdU positive by day six. Our data demonstrate that during homeostasis, the resident CD163- IM population has relatively low proliferation but exhibits some ongoing monocyte recruitment while the CD163+ IM population has low proliferation and little recruitment. LPS-induced inflammation leads to recruitment of monocytes that contribute to expansion of the CD163- IM subset and the CD163+ IM subset which then rapidly contract back to baseline.

Frances Li
Defining MARCO-virus Interactions Important for Alphavirus Clearance from the Circulation
GS3, Microbiology
Li F*, Carpentier K, Morrison T
Arboviruses, such as mosquito-borne alphaviruses, are major public health concerns, and the capacity of an arbovirus to be transmitted in a human-mosquito-human transmission cycle has fueled explosive outbreaks worldwide. Major determinants of arbovirus transmission, geographic spread and pathogenesis are the magnitude and duration of viremia in the vertebrate host. Previously, we determined that multiple

arthritogenic alphaviruses, including chikungunya (CHIKV) and Ross River (RRV) viruses, are cleared efficiently from murine circulation by scavenger receptor A6 (MARCO) expressed on liver macrophages. Utilizing an in vitro cell culture system, we uncovered that ectopic expression of MARCO promoted internalization of CHIKV and RRV particles via the scavenger receptor cysteine-rich (SRCR) domain. Further analysis of the SRCR domain from vertebrate species implicated in alphavirus transmission cycles revealed varying ability of MARCO to internalize virus particles. Collectively, these findings suggest that CHIKV and RRV particles ionically interact with the MARCO SRCR domain in a species-specific manner. Ultimately, this project may provide new insight into the molecular mechanisms that dictate arthritogenic alphavirus transmission, dissemination and pathogenesis in vertebrate hosts.

Hei-Yong (Grant) Lo
What RNAs localize to the centrosome and how do they get there?
GS3, Molecular Biology
Hei-Yong Lo*, J Matthew Taliaferro
What bulk of RNAs are present at the centrosome is unknown. The mechanisms of how certain transcripts localize to the centrosome is also unknown. I aim to understand what RNAs localize to the centrosome and the mechanisms used to localize them there.

Jacqueline Turner
Lipid signaling as a tolerogenic mechanism to modulate adaptive CD8 T cell immunity
GS2, Pharmacology
Jacqueline Turner*, Marc D'Antonio, Richard Tobin, Angelo D'Alessandro, Isabel Schlaepfer, Martin McCarter, William Robinson, Kasey Couts, Raul Torres
The mechanisms regulating CD8 T cell tolerance and dysfunction are poorly understood. We hypothesize lipid signaling is a novel mechanism of peripheral tolerance. We further hypothesize, lipid signaling metabolically reprograms CD8 T cells to regulate dysfunction. We show lysophosphatidic acid (LPA) is a signaling lipid that impairs CD8 T cell killing by inhibiting perforin degranulation. Using metabolic studies, we reveal LPA rewires CD8 T cell metabolism through rapid lipid depletion. Notably, metabolic modulators blocking mitochondrial lipid uptake abrogate LPA-induced metabolism. Experiments with receptor knock-out mice show LPAR5 is responsible for energetic bursts required for perforin degranulation. Proteomic analysis show LPA induces low-level ERK phosphorylation which is a characteristic of dysfunctional immune cells. We went on to characterize the signaling pathway and show LPA is a mechanism distinctly different from exhaustion to induce dysfunction. We further observed a reversible flux of reactive oxygen species (ROS) at physiologic concentrations. However, this flux in ROS was absent at pathologic concentrations. Interestingly, LPA is elevated in solid tumor malignancies which lead us to question if LPA is pathologically exploited by cancer. To investigate the role of LPA in cancer, we performed lipidomics on plasma samples from stage IV melanoma patients and measured LPA pre- and post-immunotherapy treatment. We found lower LPA levels predicted response to immunotherapy. Our data and others establish LPA as increasingly important mediator of tumor development and progression. In summary,

we show LPA signaling modulates CD8 T cell tolerance through metabolic reprogramming to modulate dysfunction and impair anti-tumor immunity. Our findings offer key insights into the mechanisms governing adaptive immune tolerance and identify metabolic inhibition targeting lipid signaling as a novel approach to modulate CD8 T cell immunity.

Jordan Hickman
Demystifying the effects of electrical brain stimulation on neural activity with multineuropixel recordings in mice
GS1, Neuroscience
Jordan Hickman*, Grant Hughes, Daniel Denman
Electrical brain stimulation (EBS) is used to treat a wide range of neurological and psychiatric disorders. EBS alters neural spiking and circuits and holds tremendous potential in treating disorders with aberrant neural activity. Despite this potential and its already tremendous impact in many patients, it remains unknown how EBS precisely effects neural activity, hindering its efficacy and application. Here, we propose to elucidate how e-stim alters neural activity across (i) clinically relevant e-stim parameters with (ii) cell type specificity across brain regions using high density electrophysiological recordings in mice. To achieve a three-dimensional measurements of neural activity, we will orthogonally record from three Neuropixels surrounding a stimulating electrode in multiple brain regions (visual cortex and subthalamic nucleus). This approach will provide three-dimensional high-density electrophysiological data surrounding the source of stimulation, allowing the measurement of how EBS alters the surrounding neural activity.

Joseph (Joe) Hsieh
Mechanisms of PAX3-FOXO1 Cofactors Chromatin Recruitment in Fusion-Positive Rhabdomyosarcoma
GS3, Cancer Biology
Joseph Hsieh*, Lays Sobral, Paul Jedlicka
Fusion-Positive Rhabdomyosarcoma (FP-RMS) is a highly aggressive childhood cancer. The majority of FP-RMS is driven by a PAX3-FOXO1 (P3F) oncofusion that drives disease through aberrant regulation of gene expression, promoting cell proliferation and invasion and impairing differentiation. P3F works in conjunction with cofactors such as chromatin and transcription factors to aberrantly drive transcription at target genes. The Jedlicka lab has identified a novel disease-promoting regulatory axis involving the chromatin factor KDM3A and the ETS1 transcription factor, and showed that this axis importantly contributes to P3F-dependent gene expression and phenotypes in FP-RMS. Epistatically, ETS1 is downstream of and upregulated by both P3F and KDM3A, providing a potential direct mechanistic intersection between the KDM3A/ETS1 axis and P3F in FP-RMS. In subsequent cistrome profiling, I demonstrate extensive colocalization of P3F, ETS1, and KDM3A, along with the catalytic ATPase subunit of the BAF chromatin remodeling complex BRG1, at disease-promoting genes. Further cistrome profiling following factor depletion shows that ETS1 and BRG1 positively regulate one another's chromatin recruitment, as well as that of KDM3A and P3F.

Taken together, these findings reveal important novel P3F cofactors that contribute to disease-promoting gene expression and phenotypes in FP-RMS.
I hypothesize that P3F induction of ETS1 expression promotes FP-RMS pathogenesis through increased chromatin recruitment of P3F itself and other key cofactors. The aims of this proposal are to: 1) determine whether ETS1 recruits P3F via direct protein-protein interaction, 2) define role of ETS1 T38 phosphorylation in mediating ETS1 oncogenic effects in FP-RMS, 3) determine ETS1 necessity for P3F pioneer factor effects. Further understanding of mechanisms by which cofactors interface with P3F will yield new insights into FP-RMS pathogenesis and may uncover novel treatment approaches.

Juan Santiago
Color Representation in the Primary Visual Cortex
GS2, Neuroscience
Juan Santiago Moreno*, Daniel J Denman
In visual processing, color and form are independent, sometimes redundant features that are necessary for detailed comprehension of visual scenes. Cone-opponent mechanisms from the retina reflect local spectral variation, supporting perception of color, and cone-additive mechanisms reflect local changes in luminance, supporting perception of object form. While luminance and color contrast boundaries often co-occur, the conservation of color across luminance conditions provides an indicator of material boundaries separate from illumination boundaries. In this way, color supports perception of form in ways that are complementary to luminance contrast, but the relationship between the neural activity underlying these two parallel streams in the early visual system is poorly understood. Do these systems support form perception independently, or do they feed into a common mechanism? If so, at what stage? Through the hierarchical integration of retinal afferent signals, multiple subtypes of neurons in the early visual system display varied sensitivity to spectral features, spatial features or both. How this sensitivity emerges from thalamic or intracortical inputs to primary visual cortex (V1) remains unclear. Despite differences compared to other mammalian visual systems, mice are a vital model for interrogating visual perception using genetic tools and high-resolution neural recording. In most mammals, retinal cone-opponent signals are relatively unchanged at their first synapse in the lateral geniculate nucleus of the thalamus (LGN). While mice use color discrimination in the upper visual field to guide behavior, retinal cone-opponent circuits appear insufficient for spatially localized color comparison. Recent evidence in mice has demonstrated neurons in the LGN selectively modulated by both retinal opponent mechanisms and direct cone inputs. Our own preliminary data in mice has demonstrated neurons in V1 responsive to luminance contrast in a manner dependent on the spectral content of the stimulus, suggesting thalamocortical circuits as a primary locus for integration of color and form signals. However, encoding of chromatic spatial contrast through thalamocortical circuits in remains unexplored.

Keith Dodd

Changes in functional connectivity of the striatum correlated with relative weight-gain risk of antipsychotics

GS1, Bioengineering
Dodd K*, Tregellas J, Legget K

Schizophrenia affects nearly 1% of the international population, with most patients requiring lifelong use of antipsychotics. Antipsychotics are well-known to cause various degrees of risk for weight gain, however, leading to a near two-fold increase in obesity incidence and obesity-related comorbid outcomes. The mechanisms by which antipsychotics alter the brain to cause weight gain remain unknown, making this a difficult problem to target and treat. Therefore, there is a need to better understand the antipsychotic-related functional brain changes that lead to weight gain. In this study, we begin to address this need by examining functional magnetic resonance imaging (fMRI) connectivity of the striatum, which has well-known associations with BMI and food cravings. 49 participants with schizophrenia treated with antipsychotics underwent two resting state fMRI (rs-fMRI) sessions, one before consuming a meal (pre-meal), and one after (post-meal), following one day of eucaloric energy intake and an overnight fast. Primary outcomes focused on neuronal response to a meal (i.e., pre- vs. post-meal), as our previous work has suggested this response to be aberrant in individuals with overweight/obesity. Rs-fMRI data were acquired with a 3T Siemens scanner (TE=0.3s, TR=2s, Slice Thickness=2.6mm, Spacing=3.952mm). Data were then preprocessed through the well-validated fmriprep protocol, then smoothed and denoised using the standard CONN Toolbox. All preprocessing steps were individually QC validated leading to the removal of three subjects from analysis due to QC-FC correlations falling below 95%. Seed-to-voxel analysis with a general linear model (GLM) of multiple regression was used to examine correlations between reported weight-gain risk of the antipsychotics (mild, moderate, or severe) to alterations in rs-fMRI connectivity with the striatum, pre-meal minus post-meal. Potential confounding effects were rigorously controlled for by including the following subject effects in the GLM: BMI, age, sex, and nicotine use. Multiple comparisons was stringently controlled for using standard Random Field Theory (voxel threshold: $p < 0.001$ two-sided, cluster threshold: $p < 0.05$ FDR corrected). Significant connectivity changes were observed with the left nucleus accumbens (3 clusters: p-FDR = 0.0002, 0.0056, 0.0337) and bilaterally in the caudate (left - 1 cluster: p-FDR = 0.0316; right - 2 clusters: p-FDR = 0.0035, 0.0051). For the left accumbens, cluster location and direction suggest that increased severity of antipsychotic weight gain leads to a greater drop in connectivity to regions along the bilateral postcentral gyrus and right precentral gyrus, from pre-meal to post-meal. For the left caudate, cluster location and direction suggest that increased severity of antipsychotic weight-gain leads to a smaller drop in connectivity to the left frontal pole, from pre-meal to post-meal. For the right caudate, cluster location and direction suggests that increased severity of antipsychotic weight-gain leads to a smaller drop in connectivity to the left lateral occipital cortex and superior parietal lobule, and a greater drop in connectivity to the right parietal operculum cortex, from pre-meal to post-meal. Altogether, these results suggest that antipsychotics may selectively modulate the connectivity of brain regions known to play key roles in weight regulation and food consumption.

Kelsey Kines
The role of Semaphorin 7A in aged and parous breast tissue and its role in promoting breast cancer metastases.
GS2, Cancer Biology
Abstract requested to not be included due to confidentiality disclosure.

David (Kitz) Kitzenberg
Adenosine awakens metabolism to enhance growth-independent killing of tolerant and persister bacteria across multiple classes of antibiotics
GS4, Molecular Biology
David A. Kitzenberg, J. Scott Lee, Krista B. Mills, Ju-Sim Kim, Lin Liu, Andrés Vázquez-Torres, Sean P. Colgan, Daniel J. Kao
Metabolic and growth arrest are primary drivers of antibiotic tolerance and persistence in clinically diverse bacterial pathogens. We recently showed that adenosine (ADO) suppresses bacterial growth under nutrient-limiting conditions. In the current study, we show that despite the growth suppressive effect of ADO, extracellular ADO enhances antibiotic killing in both Gram negative and Gram positive bacteria by up to five orders of magnitude. The ADO-potentiated antibiotic activity is dependent on purine salvage and is paralleled with a suppression of guanosine tetraphosphate synthesis and the massive accumulation of ATP and GTP. These changes in nucleoside phosphates coincide with transient increases in rRNA transcription and proton motive force. The potentiation of antibiotic killing by ADO is manifested against bacteria grown under both aerobic and anaerobic conditions, and it is exhibited even in the absence of alternative electron acceptors such as nitrate. ADO potentiates antibiotic killing by generating proton motive force and can occur independently of an ATP synthase. Bacteria treated with an uncoupler of oxidative phosphorylation and NADH dehydrogenase-deficient bacteria are refractory to the ADO-potentiated killing, suggesting that the metabolic awakening induced by this nucleoside is intrinsically dependent on an energized membrane. In conclusion, ADO represents a novel example of metabolite-driven, but growth-independent means to reverse antibiotic tolerance. Our investigations identify the purine salvage pathway as a potential target for the development of therapeutics that may improve infection clearance, while reducing the emergence of antibiotic resistance.

Laurel Darragh
The role of lymph nodes in priming an anti-tumor immune response both locally and distantly in head and neck cancer.
GS3, Immunology
Laurel B. Darragh, Jacob Gadwa, Tiffany Pham, Sana D. Karam
Current standard of care for HPV-unrelated oral cavity head and neck squamous cell carcinoma (HNSCC) still relies on surgery, radiation therapy, and chemotherapy. The role of lymph node treatment, via surgical dissection or elective nodal irradiation, remains controversial. We used multiple murine models of HPV-unrelated HNSCC to study the effects of neoadjuvant immunotherapy and hypofrationated radiation (SBRT), with or without elective nodal irradiation (ENI) or with or without lymph node

dissection. Effects on long-term local, regional, and distant tumor growth and metastasis were examined. We observed that mice treated with neoadjuvant SBRT without ENI, and with immunotherapy, exhibited improved local and distant tumor control and that sentinel lymph node removal reduced regional metastasis just as well as radical neck dissections. ENI reduced tumor-antigen specific T cell expansion and activation both locally and systemically. SBRT to the primary tumor also increased the activation of T cells specific to an antigen only found in the distant tumor, suggesting that this therapy can promote epitope spreading. These findings indicate that similar radical changes to the standard of care for patients with HNSCC may not only improve survival outcomes but could also reduce the extreme morbidity associated with current treatment methods.

Lily Nguyen

Combinatory epigenetic and PARPi therapy re-sensitizes resistant ovarian cancer cells to treatment and re-activates transposable elements at the transcript and epigenomic level

GS3, Molecular, Cellular, & Development Biology Lily Nguyen*, Haley Aud, Zachery L. Watson, Elizabeth Woodruff, Benjamin G. Bitler, Edward B. Chuong

Poly (ADP-ribose) polymerase inhibitor (PARPi) is one of the most effective maintenance therapies for ovarian cancer. However, the development of PARPi resistance is a growing and urgent clinical challenge. Here, we have found that an epigenetic therapy called euchromatin histone methyltransferase inhibitor (EHMTi) can re-sensitize PARPi-resistant ovarian cancer cells to PARPi, and that this combination therapy can decrease tumor growth in vitro and in vivo. To determine this mechanism of re-sensitization, we performed transcriptomic analysis (RNA-seq) and epigenomic profiling (CUT&Tag) on PARPi-resistant ovarian cancer cells. Excitingly, we found that transposable elements (TEs) are re-activated at the transcript and epigenomic level in cells treated with combinatory PARPi/EHMTi. At the transcript level, we found increased TE-derived transcripts and interferon-alpha (IFNa) signaling-related genes. Subsequent functional studies have shown necessity and sufficiency of the IFNa pathway in the re-sensitization process. These studies suggest that TE transcripts are being detected by intracellular sensors and triggering the IFNa response (i.e. viral mimicry), though this is still to be determined. At the epigenomic level, we found increased enhancer marks in several TE families including LTR10A and LTR18A. More importantly, these TE-enhancer loci correlate with the upregulation of nearby genes. Many of these corresponding genes such as TCDD inducible poly ADP-ribose polymerase (TIPARP) have known tumor suppressor functions. This suggests that some TE-derived enhancers are aberrantly silenced in PARPi-resistant cells, and that PARPi/EHMTi acts partially by re-activating these TEs and restoring expression of tumor suppressor genes. Altogether, we have evidence that combinatory EHMTi/PARPi therapy have multiple TE-mediated mechanisms by which it can re-sensitize PARPi-resistant cells and candidate genes and TEs to functionally validate. Insights gained from these studies will help develop therapeutics that can combat PARPi resistance.

Meagan Chriswell

Dual IgA/IgG family autoantibodies from individuals at-risk for rheumatoid arthritis identify an arthritogenic strain of Subdoligranulum

GS4, Immunology

Meagan Chriswell*, Jennifer Seifert, Michelle Bloom, Cliff Rims, Marie Feser, Kevin D. Deane, Jill M. Norris, Eddie James, Jane H. Buckner, William H. Robinson, V. Michael Holers, Kristine A. Kuhn

Background: Circulating autoantibodies like ACPA frequently develop years before symptoms of RA, during which the individual is at-risk for disease. Several lines of evidence suggest that these autoantibodies may be driven by microbial-mucosal interactions. We hypothesized that discrete gut-derived bacteria drive autoantibody generation.

Methods: Dual IgA/IgG family plasmablasts (n=94) were isolated from 4 individuals at-risk for RA, defined as serum anti-CCP+ (75%) and/or RF+ (75%), and from 2 anti-CCP+ individuals with early RA. Fab domains from the plasmablast-derived mAbs (PB-mAbs), selected for binding of RA-relevant antigens on a protein microarray, were expressed in a mouse IgG2a scaffold. The PB-mAbs were used to identify targeted fecal bacteria, and Ruminococcaceae(Rumino) strains were isolated from the feces of a human at-risk for RA. 16S sequencing identified seven Rumino family strains of interest, and through whole genome sequencing we identified an as yet unnamed species within genus Subdoligranulum (Sbg). Human PBMC were isolated and exposed to individual strains of Sbg to gauge memory responses by assessing in vitro CD154+ upregulation on CD4+ T cells. Germ free DBA/1 mice were colonized with each Sbg strain, as well as Prevotella copri and PBS as controls, and were monitored for stable colonization, autoantibody development, and joint swelling. Serum collected from arthritogenic Sbg monocolonized mice was injected into naive germ-free DBA/1 mice, and mice were monitored for joint swelling.

Results: All PB-mAbs bound RA-relevant antigens and 58/94 (62%) targeted families Lachno(spiraceae)/Rumino from a pool of fecal bacteria (derived from 5 healthy controls, 8 at-risk individuals, and 5 RA cases) at a disproportionately elevated level (56.31 ± 12.85% of all bacteria bound) compared to other taxa, suggesting cross-reactivity between bacterial and host antigens. We verified PB-mAb binding of Lachno/Rumino using our generated Sbg strains. We then observed an MHC class II-dependent memory T cell response in PBMCs against a subset of the Sbg strains in individuals with RA that was nearly absent in controls. To determine if Sbg could induce autoantibodies in vivo, germ-free mice were stably colonized with the Sbg strains, P. copri, or sterile PBS; within 14 days, joint swelling was observed only in mice monocolonized with a subset of the Sbg strains. Joint swelling was associated with an expansion of antibodies to RA-relevant antigens. Transfer of serum from affected Sbg strain colonized mice, but not P. copri or PBS controls, to naïve mice resulted in joint swelling.

Conclusions: A subset of circulating dual IgA/IgG PB-mAbs isolated from individuals at-risk for RA target both RA-relevant antigens and bacteria within families Lachno/Rumino. A specific strain from the genus Sbg established from an at-risk individual were recognized by T cells from RA cases. When germ-free mice were monocolonized with the specific Sbg strain, they developed joint swelling and serum autoantibodies capable of transferring the joint swelling phenotype. Our data suggests

one model in which a strain of bacteria is capable of stimulating the development of pathogenic autoantibodies.

Meghan Kellett
Investigating how nucleolar FAK drives growth and survival in thyroid cancer
GS4, Cancer Biology
MD Kellett*, V Sharma, S Sams, M Joshi, and RE Schweppe.
Late stage thyroid cancers characterized by metastasis and invasion have a poor prognosis and limited therapeutic options compared to those with localized disease. Our lab has identified Focal Adhesion Kinase (FAK) as a key regulator of thyroid cancer growth, invasion, and metastasis. FAK is a non-receptor tyrosine kinase that is auto-phosphorylated at tyrosine 397 (Y397) resulting in the activation of downstream signaling pathways. While FAK is predominantly localized at the plasma membrane, FAK has also been shown to localize to the nucleus to promote cell survival. We have found that FAK localizes to the nucleus in a subset of thyroid cancer patient tumors and that phosphorylated Y397 FAK (pY397 FAK) specifically localizes to the nucleolus. The nucleolus plays a key role in cancer progression through the synthesis of ribosomal RNA (rRNA) and subsequent increase in ribosome biogenesis, protein synthesis, and tumor growth. I hypothesize that nuclear FAK drives thyroid cancer growth and survival through phosphorylation of nucleolar proteins involved in ribosomal RNA (rRNA) transcription. First, I addressed the functional role of FAK in the nucleus and found that nuclear FAK and pY397 FAK are required for anchorage independent growth in thyroid cancer cells. Furthermore, we found that forcing FAK into the nucleus resulted in increased FAK nucleolar accumulation which was eliminated when FAK is forced into the nucleus with a non-phosphorylatable FAK mutant. These data indicate that pY397 FAK is required for FAK nucleolar accumulation. To investigate the role of pY397 FAK in the nucleolus, I performed BioID to assess protein-protein interactions and found that pY397 FAK interacts with key nucleolar proteins including NPM1, TOP1, and DDX46. Interestingly, 88% of the nucleolar proteins are involved in transcription of rRNA which is necessary for tumor growth. These data indicate that pY397 FAK interacts with key nucleolar proteins involved in rRNA transcription and that nuclear pY397 FAK drives growth and survival in thyroid cancer. Overall, nucleolar pY397 FAK may serve as a biomarker for aggressive thyroid cancer and be a novel therapeutic target.

Michael Nash
Maternal western diet programs inflammatory trained immunity in fetal and juvenile non-human primates through hematopoietic stem cells and macrophages
GS4, Integrated Physiology
*Nash MJ, Dobrinskikh E, Janssen RC, Varlamov O, Kievit P, Takahashi D, Aagaard KM, McCurdy CE, Gannon M, Jones KL, Pietras EM, Wesolowski SR, Friedman JE
Maternal western diet (mWD) exposure underlies metabolic diseases in later life, but the mechanisms by which mWD alters hematopoiesis and inflammation in the offspring beginning in the fetus are unclear. Here, we studied the consequences of mWD exposure on the metabolism, transcription, and chromatin architecture of hematopoietic stem and progenitor cells (HSPC)s and bone marrow derived macrophage (BMDM) in fetal (3rd trimester) and 3-year-old (3YO) juvenile offspring switched to a chow diet at 7

months of age (WD/CD). Fetal and 3YO HSPCs exposed to mWD had activation of NLRP3 inflammasome pathway genes and other pro-inflammatory genes, many of which are transcriptionally regulated by CEBPB and MYC, 2 key drivers of trained immunity. Fetal mWD HSPCs and 3YO WD/CD HSPCs and BMDM had elevated glycolytic activity, suggesting metabolic reprogramming in myeloid cells is inherited from hematopoietic precursors. Likewise, BMDM from 3YO WD/CD had increased pro-inflammatory gene expression, increased phagocytosis, and decreased expression of anti-inflammatory genes in response to IL4. ATAC-seq demonstrated epigenetic priming by increased accessibility of promoters on CEBPB-regulated genes, including NLRP3, in both HSPCs and BMDM from WD/CD 3YO. These results suggest that mWD exposure promotes inflammatory trained immunity beginning in fetal HSPCs that persists in 3YO HSPC and BMDM despite weaning to a healthy diet. Chromatin remodeling consistent with CEBPB and MYC activation suggests these may be pioneer factors responsible for pro-inflammatory priming of WD/CD 3YO macrophages. Thus, maternal WD may reprogram fetal development of myeloid cells to a heritable and persistent inflammatory phenotype. These changes may have long-term consequences for initiation of chronic metabolic diseases across the lifespan.

Mostafa El-Kalliny
Interrogating the role of inhibitory interneurons of the nucleus accumbens in social attachment
GS1, Molecular, Cellular, & Development Biology
Mostafa El-Kalliny*; Zoe R Donaldson
The nucleus accumbens is a region of the brain involved in forming and maintaining social attachments, the loss of which are detrimental to physical and mental health. Within the nucleus accumbens (NAc), individual medium spiny neurons, which together make up 95% of the cellular population, have been found to encode social interaction with partners. I found that individual neurons exhibit synchronous activity as a function of pair bond formation. Further analyses suggest that inhibitory interneurons, which have a powerful role in both microcircuitry function as well as stimulus-reward associations, may coordinate this synchrony between neurons. Here, I outline a set of experiments by which I will test the hypothesis that interneurons within the NAc encode and facilitate social attachment and underlying neuronal synchrony. These experiments will pave the way for investigating the mechanisms by which afferent and efferent changes at NAc interneurons facilitate social attachment, and will ultimately contribute to the development of novel, precise therapies for deficits in social bonding.

Nathaniel (Nat) Skillin
Liquid Crystal Polymers for Muscle Tissue Engineering
GS2, Chemical & Biological Engineering
Nathaniel P. Skillin*, Katie M. Herbert, Kemal A. Günay, Bruce E. Kirkpatrick, Frank W. DelRio, Kristi S. Anseth, Timothy J. White
Liquid crystals are ubiquitous in everyday life, from TV and iPhone displays to mood rings and Kevlar vests. Liquid crystallinity also naturally arises in biology across multiple length scales. On the molecular scale, solutions of procollagen fibers display liquid crystalline phases and cytoskeletal proteins within the cell can self-organize into

nematic phases. At the tissue level, dynamic epithelial monolayers can be modeled as active nematic liquid crystals, and the location and type of nematic topological defects on your finger pads differentiate your fingerprint from the other 7.9 billion humans on this planet. Molecules that can attain these liquid crystalline phases are being investigated for myriad applications including soft robotics, tunable optical devices, and more recently as biomaterials for engineered tissue culture.
We are exploring fundamental cellular behaviors such as adhesion, migration and alignment on synthetic liquid crystal polymers (LCPs). Multiple cell types exhibit the ability to adhere, migrate and proliferate on 2D LCPs without the need for cell adhesive ligands such as RGD peptides, which are required for most synthetic biomaterials. Surface-enforced alignment of nematic liquid crystal monomers or chain-extended oligomers with subsequent photopolymerization results in monodomain liquid crystal polymers that possess unique anisotropic material properties, much like muscle tissue. Indeed, C2C12 murine myoblasts grown on monodomain LCPs differentiate and fuse into myotubes which align over large length scales with the approximate direction of molecular alignment of the polymer. Furthermore, monolayers of aligned myotubes reproducibly stack in a chiral manner akin to a nematic cholesteric liquid crystal. Finally, we find that nascent deposition and remodeling of extracellular matrix proteins is an important mediator of myotube alignment and chiral stacking on these LCPs.

Nkolika Egbukichi
FAK and ECM Stiffness
GS1, Cancer Biology
Focal adhesion kinase (FAK) is a nonreceptor tyrosine kinase that is over expressed in a variety of cancers and plays an important role in cell adhesion, migration, and anchorage-dependent growth. In thyroid cancers, there is a correlation between increased FAK expression and progression to a more malignant state, consistent with the importance of signaling via mechanotransduction pathways and the progression of cancer. The role of extracellular matrix (ECM) stiffness and cellular mechanotransduction in differentiated and de-differentiated thyroid cancer is largely unknown. Our long-term goal is to identify the role of tissue microenvironment rigidity in the progression and dissemination of thyroid cancer.

Raquel Ortega
Role of chromatin on alternative end-joining and PARPi resistance
GS2, Molecular, Cellular, & Development Biology
Raquel Ortega*, Ben Bitler, Nausica Arnoult
Error-free repair of DNA breaks is critical to our species. Mutations in certain DNA repair proteins make us susceptible to highly mutagenic cancers of the ovary, breast, prostate, and pancreas[1, 2, 21, 22]. The most common mutations occur in BRCA1 and BRCA2 [9]. Current therapy of ovarian cancers in these patients includes medications called PARPi[4-6]. PARPi work by inhibiting DNA repair, including a pathway called alternative end-joining (Alt-EJ), which is only essential to these cancer cells[8, 11]. However, up to half of these patients fail to benefit from PARPi's[9]. Additionally, Alt-EJ was recently discovered, is understudied, and is becoming clinically relevant in disease. In fact, specific inhibitors of Alt-EJ are in development as promising adjuvant

therapy for PARPi-treated cancers[10, 14, 23]. These facts suggest that: 1. PARPi resistance is a growing concern and 2. We need research to understand regulators of Alt-EJ, thereby informing development of inhibitors. Preliminary data implicates higher levels of H3K9me2 with increased Alt-EJ activity. We previously described this modification as a driver of PARPi resistance, but we do not know the mechanism[12]. Therefore, I want to determine the role of H3K9me2 on the lesser-known repair pathway Alt-EJ and how this might mediate PARPi resistance. This work could help us understand histone modifications important for regulating Alt-EJ, which is understudied compared to other DNA repair pathways[16, 17]. Likewise, it can lead to a better understanding of how PARPi resistance develops in patients.

Sarah Zych

Neuromodulators independently regulate striatal dopamine and GABA co-transmission

GS5, Neuroscience

Sarah M. Zych* and Christopher P. Ford

Neuronal populations that release multiple neurotransmitters enable a dynamic range of signaling through the spatial and temporal scales of co-transmitter release. Within the basal ganglia, midbrain dopamine (DA) neurons co-release GABA from axon terminals in the striatum, facilitating learning, movement, motivation and reward. GABA co-release plays a role in addiction as reduced co-release leads to increased ethanol intake and preference in mice. However, little is known about the functional properties of co-released GABA or how it shapes DA signaling. As DA and GABA are both loaded into synaptic vesicles by the vesicular monoamine transporter, VMAT, it follows that they should be released together with the same properties. To investigate the properties of DA and GABA co-transmission, we used whole-cell voltage-clamp recordings of D2- and GABAA-receptor mediated inhibitory post-synaptic currents (IPSCs) activated by DA and GABA co-release onto striatal medium spiny neurons (MSNs). Surprisingly, we found that DA and GABA exhibit different release properties. We found that DA release is more sensitive to calcium sources and exhibits looser coupling between calcium entry and calcium sensing release machinery. DA and GABA release is also differently modulated by presynaptic GABAB, kappa opioid, and D2 receptors which all more strongly modulate the release of DA than GABA. In addition, we found that DA and GABA have different release probabilities and rely on different active zone scaffolding proteins. Genetic removal of the scaffolding protein RIM abolished the high release probability of DA, but GABA release was unaffected. Despite DA and GABA being packaged by VMAT, our data indicates that intrinsic differences exist between transmitter release, suggesting that DA and GABA are spatially segregated into different active zones with different release properties and modulation, arising from the identity of active zone proteins and synaptic organization. Understanding the mechanism and balance of DA/GABA co-transmission in normal physiology will allow us to study how these dynamics shift in disease states.

Shanawaj (Roy) Khair

Does a single ethanol exposure prior to burn injury in mice worsen pulmonary inflammation as much as episodic exposure of ethanol?

GS3, Molecular Biology

Shanawaj (Roy) Khair,* Brenda J Curtis, and Elizabeth J. Kovacs

Burn injury is a major cause of mortality and morbidity in the US and lungs are often the first organ to fail. Interestingly, patients with alcohol intoxication at the time of burn have worse clinical outcomes, including pulmonary complications. Mechanisms by which alcohol prior to burn injury leads to lung inflammation are still unclear. Previously, we reported that episodic ethanol exposure before burn in a murine model exacerbated pulmonary inflammation, with heightened lung IL-6 levels in mice. Herein, we compare episodic binge ethanol exposure before scald burn injury with a single ethanol exposure to determine if single ethanol exposure was sufficient to create a similar pulmonary response. C57BL/6 male mice were given ethanol (1.2 g/kg) or vehicle 30 min before burn; sham controls were given ethanol or vehicle. IL-6, CXCL1, and neutrophil infiltrate were measured in the lungs at 24 hours after burn and compared using Two-way ANOVA with Tukey's or Sidak's posthoc tests. Mice given burn alone had 4-fold higher lung IL-6 ($p< 0.05$) and those with a single ethanol and burn had an 11-fold increase relative to sham vehicle and ethanol mice ($p<0.05$). When we compared lung IL-6 levels the single ethanol & burn and episodic ethanol & burn groups, there were no significant differences. Next, we assessed pulmonary CXCL1 levels in the single ethanol group and found that mice with burn and ethanol had 14-fold more CXCL1 than in sham vehicle mice ($p<0.05$). Again, we did not find a difference between the single ethanol exposure and burn to those given multiday ethanol exposure and injury. Given that both CXCL1 and IL-6 are pro-inflammatory mediators, we compared the infiltration of neutrophils into lung tissue after burn and saw 4 times the number of neutrophils in burn injured mice than in sham mice ($p<0.05$) and when ethanol was present at the time of the burn, this was 6 times higher ($p<0.05$). Consistent with prior observations, when the number of neutrophils in burn and ethanol mice were compared between single and episodic ethanol exposure regimens, there was no difference. Taken together, these data show that a single exposure to ethanol prior to burn injury contributes to excessive lung inflammation as seen in episodic multiday ethanol exposure prior to burn. R35 GM131831 (EJK) and VA 1 I01 BX004335 (EJK).

Taylor Yamauchi

Investigating circuits for target acquisition

GS4, Neuroscience

Taylor Yamauchi, Jacki Essig PhD, Abigail Person PhD, Gidon Felsen PhD

The nervous system is remarkably adept at acquiring targets of interest by sorting incoming sensory information to focus on a salient target, and then orienting the body to interact with the target using a range of movements. The superior colliculus (SC), a midbrain sensorimotor structure, plays a major role in this process by selecting between targets for attention and driving the orienting movements necessary to acquire the target (i.e. eye movements, reaching). Behavioral evidence has implicated the rostral SC for controlling both small movements and movement cessation, but how the underlying circuitry accomplishes these paradoxical actions is poorly understood. Our current

hypothesis is that small amplitude movements and movement termination are controlled by distinct projection populations within the rostral SC, motor output neurons and commissural neurons respectively. Here, we leverage cutting edge circuit busting approaches in head-fixed mice performing a closed-looped virtual target acquisition task to record and manipulate specific motor output and commissural neuron populations. Consistent with both roles for driving and terminating movements, global inhibition of rostral SC neurons resulted in less accurate movement endpoints and preliminary recordings of these neurons showed increased activity during movement with a range of peak activity. Ongoing experiments target commissural or motor output populations for recording and manipulation and will further elucidate SC circuit mechanisms for target acquisition.

Thomas Forman

Investigating Srsf3-mediated alternative RNA splicing downstream of PDGFRalpha signaling in craniofacial development

GS2, Molecular Biology
Thomas E. Forman*, Katherine A. Fauntauzzo

Signaling through the platelet-derived growth factor receptor alpha (PDGFR□) plays a critical role in human and mouse craniofacial development. Phosphatidylinositol 3-kinase is the primary effector of PDGFR□ signaling during skeletal development in the mouse, leading to activation of the kinase Akt. A previous phosphoproteomic screen demonstrated that Akt phosphorylates the RNA-binding protein serine/arginine-rich splicing factor 3 (Srsf3) downstream of PI3K-mediated PDGFR□ signaling in mouse embryonic palatal mesenchyme (MEPM) cells, leading to translocation of phosphorylated Srsf3 into the nucleus. Further, ablation of Srsf3 in the murine neural crest cell lineage (cKO) results in a severe midline facial clefting phenotype due to defective proliferation and survival of cranial neural crest cells. RNA-sequencing analysis of Srsf3 cKO facial process mesenchyme identified alternative RNA splicing events enriched for transcripts encoding protein serine/threonine kinases, suggesting that alternative RNA splicing may serve as a novel feedback mechanism for intracellular kinase signaling. Here we highlight ongoing experiments to test the hypothesis that PDGFR□ signaling regulates Srsf3 protein and RNA interactions to affect the alternative splicing of transcripts necessary for craniofacial development. First, Srsf3 phosphorylation changes will be comprehensively mapped in response to PDGFR□ signaling in MEPM cells. Further, craniofacial phenotypes will be analyzed in a Srsf3 phosphomutant knock-in mouse model to determine the role of Akt-mediated phosphorylation of Srsf3 in craniofacial development. Next, Srsf3 protein interacting partners will be identified in response to PDGFR□ signaling in MEPM cells. Finally, Srsf3-RNA interactions will be purified and sequenced in response to PDGFR□ signaling in MEPM cells to identify direct targets of Srsf3 and determine if RNA binding and/or sequence specificity changes upon Srsf3 phosphorylation.

Uma Kantheti
Role of PD-L1 Signaling in Dermal Dendritic Cell Migration
GS1, Immunology
Uma Kantheti*, Erin Lucas, Beth Tamburini
Although the function of programmed death ligand 1 (PD-L1) through its interactions with PD-1 on T cells is well studied, little is understood about other extracellular binding partners of PD-L1 and the role they play in dendritic cell migration. Here, we outline a major role for PD-L1 and its binding partner CD80 in control of dendritic cell (DC) migration from the skin to the draining lymph node (dLN). Using blocking antibodies, we identify that cis binding of PD-L1 and CD80 on dermal DCs proves critical for DC migration to the dLN during inflammation. This loss of DC migration is also modulated by Tregs expressing CTLA-4, which can trans-endocytose CD80 on migratory DCs in order to prevent migration from the skin into the dLN.

Varuna Nangia
The role of non-canonical MAPK dynamics in non-genetic drug adaptation in melanoma
GS1, Chemistry & Biochemistry
TBD

William Sheeran
Neuronal ensembles underlying monogamous behavior in nucleus accumbens.
GS2, Molecular, Cellular, & Development Biology
William M. Sheeran*, David S.W. Protter, Zoe R. Donaldson
Fewer than 10% of mammalian species utilize social monogamy as a mating system. These include humans and prairie voles, but not species commonly studied in systems neuroscience such as mice and rats. Prairie voles are thus a valuable model organism for studying the neural principles of adult attachment and other complex social behaviors. Pair bonded prairie voles strongly prefer to interact with their monogamous partner and prefer to spend time in contexts previously associated with their partner. These behavioral patterns suggest that partner interaction is reinforcing for monogamous animals. Circuit-level models informed by pharmacological and genetic manipulations have highlighted the nucleus accumbens (NAc) as a crucial region for the formation and maintenance of a pair bond; the NAc integrates converging reward- and sensory-relevant information from regions such as the prefrontal cortex, ventral tegmental area, and hippocampus. However, very few studies have examined the neuronal activity dynamics involved in pair bonding in either NAc or the rest of the putative pair bond circuit. Through calcium imaging in awake, freely moving prairie voles, we have identified various activity-defined neuronal ensembles that encode crucial aspects of monogamous behavior, namely movement towards and away from pair-bonded partners or novel animals. Changes in the proportion of NAc neurons participating in partner approach ensembles over time mirrored the emergence of and predicted the strength of pair bonds. Ensembles were additionally spatially interspersed throughout NAc, mirroring the topography of social ensembles in upstream areas such as amygdala and prefrontal cortex. This signaling likely represents an important aspect of the key role of NAc in monogamous behavior and thus demands further investigation.