Vanderbilt University
and
Meharry Medical College
Departments of Pharmacology

2017 FALL PHARMACOLOGY RETREAT

Wednesday, October 25, 2017

Nelson Andrews Leadership Lodge
3088 Smith Springs Road
Nashville, TN 37013

2017 FALL PHARMACOLOGY RETREAT

Wednesday, October 25, 2017

NELSON ANDREWS LEADERSHIP LODGE
3088 SMITH SPRINGS ROAD, NASHVILLE, TN 37013

8:30 am – 9:00 am **Arrival/Registration** *(Coffee & Light Snacks available in the Great Hall)*

9:00 am **Welcome and Introductions**
J. David Sweatt, Ph.D., Chair of Pharmacology
Brittany Spitznagel, Retreat Organizing Committee

9:05 am – 10:05 am	**Scientific Session 1**	**Session Facilitator: Dave Weaver**
9:05 am – 9:20 am	Matt Wleklinski (Knollmann Lab) **"Understanding how mutations in calsequestrin-2 lead to catecholaminergic polymorphic ventricular tachycardia (CPVT)"**	
9:20 am – 9:35 am	Joe Balsamo (Hong Lab) **"Heart Failure Therapy: The Quest for New Therapeutic Targets"**	
9:35 am – 9:50 am	Kelvin Luong (Fesik Lab) **"Modulating Proteasomal Degradation of Cancer Causing Proteins"**	
9:50 am – 10:05 am	David Taylor (Kim Lab) **"Combinatorial STING and Toll-like Receptor 4 Adjuvants: A Promising Treatment to Cure Cancer"**	

10:05 am – 10:50 am **3MT®-style Presentations 1** **Moderator: Chris Hofmann; Timing: Mabel Seto**
3rd Year Pharmacology Students

Jenny Aguilar (Galli Lab)
"PIP_2 regulation of DA-associated behaviors"

Rachel Fischer (Sappington Lab)
"Altered potassium homeostasis in glaucoma and significance for retinal ganglion cell health"

Nicole Fisher (Conn & Niswender Labs)
"**mGlu7 deficiency as a novel cause of neurodevelopmental disease**"

Oakleigh Folkes (Patel Lab)
"Investigations of the underlying circuitry and molecular regulation of social interaction and anxiety"

Elizabeth Gibson (Osheroff Lab)
"*Mycobacterium* Gyrase Inhibitors" (MGIs): A Novel Class of Gyrase Poisons

James Maksymetz (Conn Lab)
"A Bottom-Up Approach to Improve Top-Down Processing: The Role of M_1 Muscarinic Receptors in the Prefrontal Cortex and Schizophrenia"

Rafael Perez (Winder Lab)
"Norepinephrine's yin and yang: opposing roles of adrenergic alpha$_{2a}$- and beta$_2$- receptors in stress-induced relapse of drug seeking"

Nicole Perry (Gurevich & Iverson Labs)
"A signaling conundrum: the interaction of arrestin-3 with MAP kinases"

FJ Prael (Weaver Lab)
"Chemical Genetic Determination of the Effect of Cation Chloride Cotransporter Potentiation on Epilepsy"

10:50 am	**Break!!**

10:55 am – 11:55 am	**Scientific Session 2**	**Session Facilitator: Seva Gurevich**
10:55 am – 11:10 am	Jordan Brown (Winder & Sweatt Labs) **"DNA methylation regulates neuronal intrinsic excitability through altered gene expression of calcium-gated potassium channels"**	
11:10 am – 11:25 am	Ben Coleman (Sweatt Lab) **"Regulation of *Arc* Expression via DNA Methylation"**	
11:25 am – 11:40 am	Brynna Paulukaitis (Sweatt Lab) **"The Role of Histone MacroH2A Subunit Exchange in Memory Consolidation"**	
11:40 am – 11:55 am	Nathan Winters (Patel Lab) **"Endocannabinoid Regulation of Amygdala Circuits in Alcohol Use Disorders"**	

11:55 am	**Group Photo**
12:00 pm – 1:00 pm	**Lunch Break**

1:00 pm – 1:45 pm	**Scientific Session 3**	**Session Facilitator: Brian Wadzinski**
1:00 pm – 1:15 pm	Sheryl Vermudez (Niswender & Conn Labs) **"Metabotropic glutamate receptor 3 as a therapeutic target for cognitive phenotypes in Rett syndrome"**	
1:15 pm – 1:30 pm	Annah Moore (Niswender & Sweatt Labs) **"Muscarinic Acetylcholine Receptor 4 (M_4) as a Novel Therapeutic Target for Pitt Hopkins Syndrome"**	

1:30 pm – 1:45 pm	Jamal Bryant (Blind Lab) **"SF-1 regulation by the acyl chains of phospholipids"**

1:45 pm – 2:30 pm **3MT®-style Presentations 2** **Moderator: Krystian Kozek; Timing: FJ Prael**
2nd Year Pharmacology Students

Mark Crowder (Blind Lab)
"Characterizing a role for IPMK, a lipid kinase, in liver physiology"

Andrew Feigley (Davies Lab)
"Investigating the possible mechanisms of N-acylethanolamines (NAEs) that alter weigh gain and appetite"

Eric Figueroa (Denton Lab)
"Molecular pharmacology of swelling-activated LRRC8 anion channels"

Breanne Gibson (Schoenecker Lab)
"Targeting the acute phase response to improve tissue repair following traumatic injury"

Chris Hofmann (Emeson Lab)
"Functional consequences of RNA editing on $mGlu_4$ dimerization"

Corey Seacrist (Blind Lab)
"Exploring the Signaling Network of Inositol Polyphosphate Multikinase"

Mabel Seto (Lindsley Lab)
"Effort toward probe development for the metabotropic glutamate receptor type 7 ($mGlu_7$)"

Kayla Shumate (Emeson Lab)
"CAPS1 RNA editing: a modulator of synaptic transmission"

Brittany Spitznagel (Weaver Lab)
"Selective Slack Modulators for the Treatment of Rare Childhood Epilepsy"

2:30 pm – 3:30 pm	**Poster Session**
A-1	Kristopher Abney, MMC **"ACTIVATION OF G PROTEIN-GATED INWARDLY-RECTIFYING POTASSIUM (GIRK) CHANNELS AS A NOVEL THERAPEUTIC TARGET FOR PAIN"**
A-2	Lillian Brady, Postdoctoral Fellow (Hamm Lab) **"The role of complexin on Gβγ-SNARE interaction and the regulation of exocytosis by $G_{i/o}$-coupled G-protein coupled receptors"**

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A-3	Claire DelBove (Zhang Lab) "**Gamma-secretase and cholesterol modulates neuronal surface expression of full-length amyloid precursor protein**"
A-4	Yuanjun Guo (Lal Lab) **"Essential Role of HIPK2 in Maintaining Basal Cardiac Homeostasis"**
A-5	Kenneth Harris, MMC (Ramesh Lab) **"SEX-SPECIFIC DIFFERENCES IN BENZO(A)PYRENE [B(a)P]-INDUCED COLON CARCINOGENESIS"**
A-6	Danyeal Heckard, MMC **"Selective activation of membrane estrogen receptors decrease GIRK channel function to rapidly attenuate opioid receptor-like 1 (ORL1) receptor-mediated modulation of nerve injury-induced tactile hypersensitivity"**
A-7	Hussain Jinnah (Emeson Lab) **"RNA Editing-Mediated Regulation of Serotonin 2C Receptor Expression"**
A-8	Krystian Kozek (Weaver Lab) **"Discovery and Development of Tools to Study the Role of G Protein-gated Inwardly-rectifying Potassium (GIRK) Channels in Addiction"**
A-9	Sudan Loganathan, MMC **"Epigenetic-based Combination Therapy for the Treatment of Ewing Sarcoma"**
A-10	Stephanie Moore (Schoenecker Lab) **"Traumatic Skeletal Muscle Calcification: A Balance of Pyrophosphate and Inorganic Phosphate"**
A-11	Shan Parikh (Knollmann Lab) **"T3+Dex generates functional t-tubules in hiPSC-CM"**
A-12	Kristin Peterson (Hasty Lab) **"Novel regulation of insulin receptor processing & signaling by complement factor 5"**
A-13	Aparna Shekar (Galli Lab) **"A rare, autism-associated in-frame deletion in the dopamine transporter exhibits profound functional deficits"**
A-14	Megan Shuey (Brown Lab) **"Differences in the Treatment of Resistant Hypertension in African Americans and European Americans in a Clinical Setting"**
A-15	Ebrahim Tahaei (Elefteriou Lab) **"The reduced osteogenic differentiation potential of *Nf1*-deficient osteoprogenitors is TGFβ and EGFR-independent"**
A-16	Emily Warren (Konradi Lab) **"Decreased mtDNA decreases mitochondrial creatine kinase: Energetic instability in L-DOPA Induced Dyskinesia"**
A-17	David Wooten and Christian Meyer (Quaranta Lab) **"Quantifying drug synergy along axes of potency and efficacy"**

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A-18	YunYoung Yim (Hamm Lab) **"G protein specificity of inhibitory adrenergic α_{2a} receptor mediated modulation of synaptic transmission and SNARE"**
A-19	Yuantee Zhu (Elefteriou Lab) **"Tissue-specific role of norepinephrine transporter in age-related bone loss"**

3:30 pm	**Announcements and Prizes Awarded**

4:00 pm

Keynote Address by Mark Wallace, Ph.D.

"A New Vision for Graduate Education at Vanderbilt"

Introduction by Kristin Peterson, Graduate Student, Alyssa Hasty's lab

5:00 pm – 7:00 pm **Social Time**

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ABSTRACTS

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Understanding how mutations in calsequestrin-2 lead to catecholaminergic polymorphic ventricular tachycardia (CPVT)

Matthew Wleklinski, Shan Parikh, Bjorn Knollmann
Department of Pharmacology
Vanderbilt University School of Medicine, Nashville, TN 37232

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a genetic arrhythmia that is characterized by syncope and cardiac arrest occurring during exercise or emotional stress. The pathogenesis of this arrhythmia is thought to occur from mutations in genes that code for channel-proteins responsible for regulating cardiac electrical function. The most common proteins involved in CPVT are the ryanodine receptor (RyR2) and calsequestrin (Casq2). These proteins are vital to the excitation-contraction (EC) coupling cycle (Fig. 1). EC coupling is driven by calcium influx via voltage-gated calcium channels. The majority of calcium is stored within the sarcoplasmic reticulum (SR) and is released during excitation through RyR2 after calcium is sensed by the receptor. In the SR, Casq2 is the major calcium binding protein and helps to regulate calcium levels during and after EC coupling.

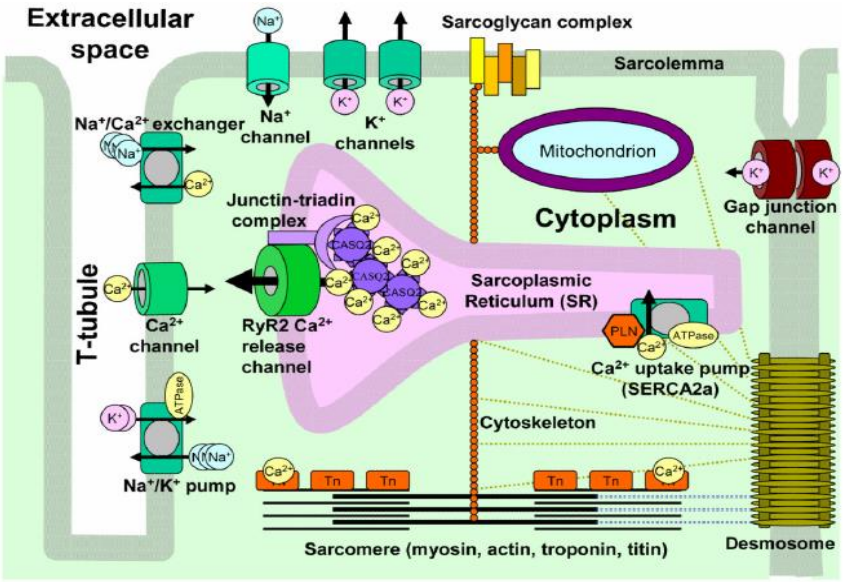

Figure 1.
Cardiac myocyte. Intracellular organelles and protein complexes involved in EC coupling.

Research has shown that mutations within Casq2 lead to a depletion of the protein within the SR. This results in an inability to buffer calcium levels during EC coupling. When the heart is stimulated, spontaneous calcium release occurs and can cause CPVT. Up until 2016, all mutations within Casq2 displayed an ***autosomal recessive*** inheritance. A recent study was conducted in a family with an ***autosomal dominant*** inheritance of CPVT that lead to the discovery of a novel missense mutation within Casq2 at amino acid 180 changing a lysine to an arginine (K180R). This was the first time an autosomal dominant mutation had been found in calsequestrin. Initial studies have shown that mice that have the K180R mutation have a CPVT phenotype when stimulated with isoproterenol, and cellular studies have shown that protein levels of calsequestrin are normal compared to wild type cells, suggesting that calcium handling is altered through a different mechanism. Protein analysis places this mutation within the SR junctional face membrane interaction domain of Casq2. **This leads to the hypothesis that the K180R mutation in Casq2 induces CPVT by affecting the proteins ability to interact with RyR2 leading to spontaneous release of calcium.** The goal of this project is to investigate how this mutation affects Casq2 function, SR calcium handling, and triggers CPVT.

Heart Failure Therapy: The Quest for New Therapeutic Targets

Joseph A. Balsamo[a], Charles H. Williams[b], Tromondae K. Feaster[a], Jonathan E. Hempel[c], and Charles C. Hong[a,b,c].

[a] *Department of Pharmacology, Vanderbilt University, Nashville, TN 37232, USA.*

[b] *Division of Cardiovascular Medicine, Vanderbilt University, Nashville, TN 37232, USA.*

[c] *Vanderbilt Institute of Chemical Biology, 896 Preston Research Building, Vanderbilt University, Nashville, TN, 37232, USA.*

Heart failure (HF) is a leading cause of mortality in the US, killing 48% of patients within five years of initial diagnosis. Historically, HF therapeutic strategy involved efforts to enhance myocardial performance by increasing cellular cyclic AMP (cAMP) through β-adrenergic receptor (β-AR) agonism or phosphodiesterase (PDE) inhibition. Unfortunately, chronic elevation of cellular cAMP drives maladaptive changes such as calcium handling perturbations, which result in HF progression, arrhythmia and sudden death. Consequently, neurohormonal stimulation is now contraindicated in HF, long term; but rather current HF therapies are focused on neurohormonal blockade aiming to blunt HF progression. From HF drug discovery perspective, however, decoupling of drugs targets to enhance acute cardiac performance from those to improve long-term HF outcomes have meant that development of new HF drugs requires expensive long-term outcome studies without short-term benchmarks of efficacy.

The strategy in the Hong lab is to identify novel HF therapeutic strategies to both improve short-term cardiac performance and long-term outcomes. Currently, the Hong lab employs a human induced pluripotent stem cell cardiomyocyte (hiPSC-CM) model to identify novel compounds and targets that enhance hiPSC-CM function. Recently, we identified a small molecule, Eggmanone (EGM), as a therapeutic candidate that enhances contraction and relaxation dynamics without compromising calcium handling in hiPSC-CMs. We then probed EGM's biological targets with a chemically modified EGM amenable to proteomics pulldown. Coupling this approach with mass spectrometry (MS) we identified farnesyl diphosphate synthase (FDPS) as a potential target. Interestingly, over-expression of FDPS in mouse is associated with heart failure, and a human phenome-wide association study (PheWAS) with the BioVU database indicates associations between several single nucleotide polymorphisms (SNPs) in the FDPS gene and altered risks of developing HF. Based on these results, we hypothesize that inhibition of FDPS, through pharmacological intervention or naturally occurring polymorphisms, enhances acute hiPSC-CM performance. Using the combined chemical biologic and genetic strategies, we hope to identify therapeutic targets that enhance both acute cardiac performance and improve long-term HF outcomes, thereby de-risking subsequent HF drug development efforts.

Modulating Proteasomal Degradation of Cancer Causing Proteins

Kelvin H. Luong[1], Bin Zhao[2], William G. Payne[2], Benjamin P. Kleinfelter[2], Jason Phan[2], Edward T. Olejniczak[2], Stephen W. Fesik[1,2]
[1]Department of Pharmacology, [2]Department of Biochemistry
Vanderbilt University School of Medicine, Nashville TN 37232, U.S.

Of all known human proteins, roughly 20% are “druggable” in the sense of targeting their catalytic and allosteric sites. However, many prevalent diseases like cancer stem from protein-protein interactions (PPIs) that cannot be drugged in this manner. Proteolysis-targeting chimeras (PROTACs) have emerged as a powerful new method to drug these targets by redirecting members of a cell’s E3-ubiquitin targeting system. PROTAC molecules bring a target protein in close proximity to the E3 ligase, marking the target for degradation by attaching a polyubiquitin chain. These PROTACs are composed of one ligand for the target protein and one for the E3 ligase, connected by a linker. Currently, only two E3 ligases are used in this strategy. We seek to increase knowledge in this premature field by examining novel E3-ubiquitin ligase recruitment, a core component of PROTAC technology. We have conducted initial HSQC-NMR experiments to show our expressed E3 ligase constructs are suitable for our fragment-based approach in designing tight-binding ligands.

Combinatorial STING and Toll-like Receptor 4 Adjuvants: A Promising Treatment to Cure Cancer

David R. Taylor

Immunotherapy has been emerging as a promising approach to treat cancer because of the endogenous specificity of our immune system as well as the inherent memory of our adaptive immune system against “foreign” malignant cancer cells while minimizing toxicities. Among these therapies, Toll-like receptor 4 (TLR-4) adjuvants have been well characterized and there are now multiple GMP grade agents that have gone into humans. However, almost all TLR4 monotherapies or adjuvants have failed in clinical trials. To solve this problem, we are combining TLR-4 and Stimulator of Interferon Genes (STING) receptor adjuvants because they have different modes of activation and Dr. Kim, with the help of his collaborators, have formulated a STING adjuvant (RR-S2 CDA) that was shown to have potent anti-tumor effects in-vivo. To test this, we will utilize both in-vitro and in-vivo models assessing the combinatorial effects on antigen presenting cell activation, t-cell priming response, and most importantly an anti-tumor response. Executing this will provide possible knowledge of how this novel combination will translate in the clinic and further improve upon immunotherapy efficacy.

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FJ Prael (Weaver Lab)
"Chemical Genetic Determination of the Effect of Cation Chloride Cotransporter Potentiation on Epilepsy"

Jenny Aguilar (Galli Lab)

"PIP_2 regulation of DA-associated behaviors"

Amphetamine (AMPH) and its derivatives are one of the most commonly abused prescription drugs by teens and young adults. AMPH principally mediates its physiological and behavioral effects by elevating extracellular dopamine (DA). DA homeostasis is maintained by the DA transporter (DAT), a presynaptic membrane protein that mediates the high-affinity reuptake of released DA from the synaptic cleft. AMPH induces N-term phosphorylation of the DAT, which leads to transport-mediated efflux of DA. We have previously demonstrated that phosphatidylinositol (4,5)-bisphosphate (PIP_2) directly interacts with the DAT, and facilitates AMPH-induced DA efflux. PIP_2 is the phospholipid precursor of the second messengers inositol trisphosphate (IP_3), diacylglycerol (DAG), and phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), and is, itself, a second messenger and cofactor that regulates protein function. We utilize a combination of biochemistry, electrophysiology and computational modeling to elucidate further the molecular mechanisms through which PIP_2 supports DA efflux through the DAT. Finally, using a behavioral model we developed in *Drosophila melanogaster*, we determine whether PIP_2 modulates DA-associated behaviors. To our knowledge, we are the first to demonstrate that DAT interacts with membrane lipids to regulate AMPH-induced DA efflux, and DA-associated behaviors.

Rachel Fischer (Sappington Lab)

"Altered potassium homeostasis in glaucoma and significance for retinal ganglion cell health"

Vision loss in glaucoma is caused by the degeneration of retinal ganglion cells and their axons that form the optic nerve. This degeneration occurs in a heterogeneous pattern across the retina. Glaucoma produces functional deficits in RGCs prior to structural degeneration. There is a potential therapeutic window after the onset of functional deficits, but prior to irreversible, physical loss of RGC axons in which axonopathy could be interrupted and further vision loss prevented. We are examining whether alterations in the cation homeostasis of the retina could be impacting RGC physiology and heterogeneous degeneration during glaucoma.

Nicole Fisher (Conn & Niswender Labs)

"**mGlu7 deficiency as a novel cause of neurodevelopmental disease**"

For patients, a diagnosis is the first step towards treatment. But what happens when a diagnosis can't be reached? Luckily, advances in DNA sequencing technology have allowed scientists to look deeper into the genome for causes of disease. My thesis focuses on mutations recently identified in patients with undiagnosed brain disorders the lie within a receptor known as mGlu7. Our lab has previously found that mGlu7 is involved in a similar disorder known as Rett syndrome. My current research aims to validate that patient-derived mutations lead to loss of receptor function and that loss of receptor function in mice mimics the human condition. Our ultimate goal is to "fix" broken mGlu7 receptors with small molecules that may one day be developed into novel drugs.

Oakleigh Folkes (Patel Lab)

"Investigations of the underlying circuitry and molecular regulation of social interaction and anxiety"

Social interaction deficits and anxiety are highly comorbid symptoms in several psychiatric illnesses including autism spectrum disorder (ASD). Two brain regions that are involved in the regulation of these two behaviors are the basolateral amygdala (BLA), a well-established regulator in the development of anxiety and social interaction, and the nucleus accumbens (NAc), a critical region for regulation of reward and motivation. We have used *in vivo* optogenetics to assay the role of the BLA-NAc circuit in social interaction and anxiety-like behaviors in a mouse model. Our research demonstrates that activation of this pathway at 20hz and 5hz stimulation patterns causes a decrease in social interaction, as assayed by the three chamber social interaction task, and no effect on anxiety-like behavior. Additionally, our data show the glutamatergic BLA-NAc circuit is sensitive to endogenous cannabinoid (eCB) signaling. These findings could be relevant for elucidating pathophysiological mechanisms and therapeutic approaches for the treatment of psychiatric disorders characterized by increased anxiety and social dysfunction including ASD.

Elizabeth Gibson (Osheroff Lab)

"*Mycobacterium* Gyrase Inhibitors" (MGIs): A Novel Class of Gyrase Poisons

Mycobacterium tuberculosis, the disease-causing organism of tuberculosis (TB), is one of the leading causes of mortality worldwide. About one-third of the world's population has latent TB and millions contract this infection every year. Due to the rise of resistance and difficulty in treatment, TB has become a global health priority of the World Health Organization. Thus, there is an urgent need for drug discovery and development of anti-tubercular drugs to combat this worldwide problem. A novel drug class, *Mycobacterium* gyrase inhibitors have been developed, which acts on an already validated drug-target, type II topoisomerases. Understanding the mechanism of which this class interacts with its target, the mechanism of which it kills the cell, and the effect of these compounds on resistant strains of TB will benefit future development of this and other drug classes.

James Maksymetz (Conn Lab)

"A Bottom-Up Approach to Improve Top-Down Processing: The Role of M_1 Muscarinic Receptors in the Prefrontal Cortex and Schizophrenia"

Dysfunction of the prefrontal cortex (PFC) is a core feature of schizophrenia (SCZ) and contributes to deficits in working memory and executive function. These cognitive deficits are not treated by current antipsychotic medications and prevent many patients from fully integrating back into society. In the search for novel targets for the treatment for cognitive deficits in SCZ, the M_1 muscarinic receptor has emerged as a leading candidate. Our lab and others have developed selective M_1 positive allosteric modulators (PAMs) that have shown efficacy in preclinical animal models but how this efficacy is achieved is still unclear. A constant improvement in our understanding of the molecular and circuit-level function of the M_1 receptor in the PFC is vital to the continued development of M_1 PAMs. With this improved understanding, we can develop M_1 PAMs with the optimal pharmacodynamic properties to ultimately translate to humans and improve cognition and lives of those suffering from schizophrenia.

Rafael Perez (Winder Lab)

"Norepinephrine's yin and yang: opposing roles of adrenergic alpha$_{2a}$- and beta$_2$- receptors in stress-induced relapse of drug seeking"

Addiction is a devastating disease that has a monumental human and economic impact, due to the propensity for relapse. Since stress is often cited as a precipitating factor for craving and relapse, norepinephrine receptor ligands that reduce stress, have been investigated as potential therapies for addiction. Adrenergic alpha$_{2a}$-receptor agonists and beta$_2$-receptor antagonists have shown modest success at reducing anxiety and craving in patients. Understanding how these receptors modulate the activity of stress-processing brain regions is critical for the development of more efficacious therapies. We are addressing this challenge using a combination of behavioral, pharmacological and molecular approaches to determine the mechanisms underling the opposing effects of alpha$_{2a}$- and beta$_2$-receptor ligands on craving and relapse.

Nicole Perry (Gurevich & Iverson Labs)

"A signaling conundrum: the interaction of arrestin-3 with MAP kinases"

Mitogen-activated protein kinase (MAPK) cascades regulate cellular pathways via activation of three sequentially acting kinases. Scaffold proteins facilitate activation of these three-tiered cascades primarily through subcellular localization of the kinase components. Arrestins scaffold several MAPK cascades, leading to activation of the final effector kinase and proper biological response. The mechanisms that underlie MAPK activation via arrestin have yet to be elucidated; however, the interaction is likely influenced by the conformation and activation state of the kinase. My thesis work sheds light on how arrestin-3 mechanistically scaffolds several kinase cascades and reveals that the interaction is a complex, kinase-dependent process.

FJ Prael (Weaver Lab)

"Chemical Genetic Determination of the Effect of Cation Chloride Cotransporter Potentiation on Epilepsy"

Epilepsy is a chronic and debilitating neurological disorder that is predicted to afflict 1 in 26 people at some point in their life. Despite the advent of modern antiepileptics, nearly one-third of epileptic patients do not respond to any available treatment. Novel approaches to the treatment of epilepsy are therefore urgently needed. Potentiation of cation chloride cotransporter (CCC) activity is an attractive antiepileptic strategy. A plethora of evidence links deficits in CCC function to seizure activity in animal models and in the clinic. However, there is currently no pharmacological evidence illustrating that CCC potentiation attenuates seizures. One reason for this paucity of evidence is a lack of adequate pharmacological probes that potentiate CCC activity. Current CCC potentiators lack specificity, potency, a known mechanism of action, or desirable pharmacokinetics. Through my thesis project, I will discover new pharmacological potentiators of CCC activity, investigate the effect of pharmacological potentiation of CCC in an epilepsy model, and investigate fundamental mechanisms of CCC activation. The data I will generate may facilitate the development of a novel approach to the treatment of millions of patients afflicted with epilepsy.

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DNA methylation regulates neuronal intrinsic excitability through altered gene expression of calcium-gated potassium channels. *Jordan A. Brown[1], Garrett Kaas[2,3], Jane Wright[2], Celeste Greer[2], Danny G. Winder[1,2,3,4] and David Sweatt[1,2,3].*

Departments of Pharmacology[1], Molecular Physiology and Biophysics[2], Vanderbilt University, Nashville, TN, USA. [3]Vanderbilt Brain institute, Vanderbilt University, Nashville, TN. Vanderbilt Center for Addiction Research, Vanderbilt University, Nashville, TN.

Learning and memory are dependent upon activity-regulated neuronal plasticity. Integration and storage of these memories involve alterations in synaptic strength and membrane excitability regulated by changes in gene expression. Epigenetic modifications, including DNA methylation, have been suggested as possible mechanisms for this regulation of gene expression. Recent studies support DNA methylation as a regulator of site-specific synaptic plasticity and neuron-wide synaptic scaling. However, an association between DNA methylation and an additional form of synaptic plasticity, intrinsic membrane excitability, is less defined. We hypothesize that epigenetic mechanisms are involved in the regulation of gene expression of these voltage- and calcium-gated ion channels. Alterations in intrinsic excitability have been associated with several neurological and neuropsychiatric disorders including anxiety, epilepsy, drug addiction and depression.

Our preliminary studies show that inhibition of DNA methyltransferases (DNMTs), the enzymes that catalyze DNA methylation, results in increased neuronal intrinsic excitability measured with whole cell patch-clamp electrophysiology. Increased intrinsic excitability occurred with pharmacological inhibition of DNMT using a small molecule competitive antagonist as well as with an antisense oligonucleotides (ASO)-mediated knockdown of DNMT. To elucidate on the role of DNA methylation mediated regulation of intrinsic excitability, we will design Transcription Activator Like Effectors (TALE) and CRISPR-dCas9 constructs to artificially change SK channel gene expression in primary cortical neurons. Attaching the catalytic domain of DNMT and TET1, the enzyme responsible for DNA demethylation, to our TALE and CRISPR constructs will allow us to selectively methylate and demethylate SK channels genes, respectively. Altered methylation levels will be confirmed using bisulfite sequencing and methylated DNA immunoprecipitation (MeDIP). We will determine alterations in intrinsic membrane excitability following methylation-mediated changes in SK channel gene expression using whole cell patch-clamp electrophysiological recordings. Next, packaging these constructs in Adeno-Associated Viruses (AAVs) will allow for direct infusion into the mouse hippocampus. We will use a battery of behavioral paradigms, including elevated plus maze, object location memory, and fear conditioning, to determine the effects of DNA methylation of these SK channels on cognitive function including changes in learning and memory.

Regulation of *Arc* Expression via DNA Methylation

Ben C. Coleman, Garrett A. Kaas, John D. Sweatt
Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232

Our understanding of the neuronal mechanisms that lead to the formation of a memory has grown significantly over the past few decades. An underlying mechanism of memory formation is homeostatic plasticity, which is defined as the ability of a neuron to regulate its excitability at the whole cell level to maintain signal specificity. Synaptic scaling, or increasing or decreasing the strength of a synapse by changing the number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) present on the post-synaptic neuron, is one way neurons accomplish homeostatic plasticity. The activity-regulated cytoskeleton-associated (Arc) protein is critical to synaptic downscaling through its control of AMPAR endocytosis during periods of neuronal activity. *Arc* is an immediate early gene, tightly regulated by neuronal activity and essential for synaptic plasticity and memory. DNA methylation has recently emerged as an exciting and reversible mechanism cells use to "turn on or off" specific genes. With Arc playing an imperative role in synaptic downscaling and recent evidence for the role of DNA methylation in homeostatic plasticity, I propose *Arc* as a novel target for epigenetic regulation via DNA methylation.

To first validate that DNA methylation may play a role in the transcription of *Arc*, I will stimulate mouse primary neurons and measure the changes in methylation of *Arc*. We predict that the stimulated neurons will have decreased *Arc* methylation compared to non-stimulated cells. In order to directly alter the methylation of *Arc*, we are developing vectors to express transcription activator-like effectors (TALEs) targeted to the promoter region of *Arc* that are attached to either a ten-eleven translocation (TET) or DNA methyltransferase (DNMT) enzymatic domain to specifically de-methylate or methylate *Arc*, respectfully. Preliminary data gathered from Neuro 2a cells show the TALEs can target specific sequences in the genome. We predict that the TET-containing TALE will lead to an increase in *Arc* transcription and that the DNMT-containing TALE will lead to a decrease in *Arc* transcription, as compared to non-transfected cells. I will then perform electrophysiology experiments on mouse primary neurons that are transfected with either TALE to determine how the methylation of *Arc* impacts synaptic scaling. We predict that cells transfected with the TET-containing TALE will exhibit increased synaptic downscaling, and the opposite to be true for cells transfected with the DNMT-containing TALE. If these predictions are found to be true, it will greatly enhance our understanding of the mechanism behind the rapid transcriptional regulation of *Arc*, a gene vital for learning and memory. Moreover, success in these initial experiments will set the stage for subsequent studies *in vivo* to directly investigate the role of arc gene methylation in behavioral memory formation and storage.

The Role of Histone MacroH2A Subunit Exchange in Memory Consolidation

Brynna S. Paulukaitis, Iva B. Zovkic♠, and J. David Sweatt**
**Department of Pharmacology, Vanderbilt University School of Medicine, Nashville TN 37232*
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Few therapeutic options exist for intellectual and cognitive disorders, despite their drastic impact on quality of life of both the affected individuals and their caregivers. Epigenetic mechanisms, including histone subunit exchange, have recently emerged as critical regulators of cognition, opening up a new realm of therapeutic targets for these disorders. We, and others, have recently discovered that histone variants are exchanged in neuronal chromatin during memory consolidation. Only a few histone variants have been studied as they relate to learning and memory in the central nervous system, but to date, we know that nucleosomal H3.3 incorporation and H2A.Z eviction are necessary for memory formation in mice. We are particularly interested in a novel histone variant that has yet to be studied in the brain—macroH2A. MacroH2A is traditionally known for its enrichment on the inactive X chromosome in females, suggesting it maintains chromatin in an inactive state. As both expression of activity genes and repression of memory-suppressor genes are important for memory formation, we want to investigate whether macroH2A exchange mediates memory formation by altering the transcriptional state of neuronal chromatin.

Preliminary data from the Zovkic laboratory has shown that virally-mediated knockdown of macroH2A in the mouse hippocampus impairs contextual fear memory. Thus, we hypothesize that contextual fear training establishes memory via subunit exchange of the histone variant macroH2A within nucleosomes. Given macroH2A's traditionally repressive nature, we propose that macroH2A mediates memory formation by binding and repressing the function of memory-suppressor genes. To test this, we will examine macroH2A subunit exchange in mice in response to contextual fear conditioning by measuring changes in macroH2A expression and macroH2A binding to memory-associated genes that occur after training. We will determine the necessity of macroH2A for memory by knocking out macroH2A in mice and evaluating for memory deficits, and we will also evaluate fear memory in mice over-expressing macroH2A.

Endocannabinoid Regulation of Amygdala Circuits in Alcohol Use Disorders

Nathan D. Winters[1] and Sachin Patel[1,2]
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Alcohol use disorders (AUDs) are characterized by compulsive alcohol consumption and a shift from use to dependence, driven by the need to consume alcohol to ameliorate associated negative affective states, such as anxiety and depression. Negative affective states associated with AUDs have long-lasting effects throughout abstinence, are major drivers of relapse, and are intractable to current therapies for these conditions. Affecting approximately 30% of the US population, AUDs are among the most prevalent mental disorders in adults. Given the incidence and lack of effective therapies for AUDs, these challenges warrant investigation into novel treatment mechanisms for managing AUD-associated negative affective states.

Increased activity in the extended amygdala (EA) and insular cortex have been implicated in the generation of negative affects associated with addiction, including during alcohol withdrawal. Given that EA neurons receive extensive input from the insular cortex, this suggests that hyperactivation of the EA through increased glutamate release from insular cortex afferents may play a role in negative affect generation. Excitatory inputs into the EA are extensively regulated by the endocannabinoid (eCB) system, suggesting that eCBs may be a key regulator of alcohol withdrawal-related anxiety.

eCBs are bioactive signaling lipids that are synthesized within the postsynaptic compartments of neurons and are released as retrograde messengers that act to reduce presynaptic neurotransmitter release probability. The eCB system has been implicated in the regulation of stress and anxiety-like states, including alcohol withdrawal. We will be characterizing the role of the major brain eCB, 2-arachidonoylglycerol, in regulating insular cortex glutamatergic afferents onto physiologically distinct subpopulations of neurons in the EA, defined by their expression of the stress-related neuropeptides, corticotropin-releasing factor and somatostatin. Additionally, we will determine the physiological adaptations at these synapses and their eCB signaling properties that occur during protracted withdrawal from chronic alcohol consumption. These studies will provide novel insight into the neurobiological mechanisms underlying negative affect generation associated with AUDs, as well as explore the therapeutic potential of developing 2-AG-based therapies for treating these disorders.

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Wednesday, October 25, 2017

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1:00 pm – 1:45 pm	**Scientific Session 3**	**Session Facilitator: Brian Wadzinski**
1:00 pm – 1:15 pm	Sheryl Vermudez (Niswender & Conn Labs) **"Metabotropic glutamate receptor 3 as a therapeutic target for cognitive phenotypes in Rett syndrome"**	
1:15 pm – 1:30 pm	Annah Moore (Niswender & Sweatt Labs) **"Muscarinic Acetylcholine Receptor 4 (M_4) as a Novel Therapeutic Target for Pitt Hopkins Syndrome"**	
1:30 pm – 1:45 pm	Jamal Bryant (Blind Lab) **"SF-1 regulation by the acyl chains of phospholipids"**	

Metabotropic glutamate receptor 3 as a therapeutic target for cognitive phenotypes in Rett syndrome

Sheryl Anne D. Vermudez[1,2], Rocco G. Gogliotti[1,2], Nicole M. Fisher[1,2], Branden Stansley[1,2], Craig W. Lindsley[1,2,3], P. Jeffrey Conn[1,2,4], and Colleen M. Niswender[1,2,4]

[1]Department of Pharmacology, [2]Vanderbilt Center for Neuroscience Drug Discovery, [3]Department of Chemistry, Vanderbilt University, Nashville, TN 37232

[4]Vanderbilt Kennedy Center, Vanderbilt University Medical Center, Nashville, TN 37232

Loss-of-function mutations in the transcription factor *methyl-CpG-binding protein 2* (*MeCP2*) lead to the X-linked neurodevelopmental disorder, Rett syndrome (RTT). Affecting about 1 in 10,000 females, RTT is characterized by a spectrum of phenotypes including deficits in motor, cognition and social abilities. Despite the exciting finding that phenotypes can be rescued in mouse models of the disease by normalizing MeCP2 gene dosage, a narrow therapeutic window presents challenges, as a 0.5x over-expression of MeCP2 can cause deleterious effects. An alternative approach to therapeutic development is to target MeCP2-regulated genes involved in neurotransmission, such as the metabotropic glutamate receptors. We have found that metabotropic glutamate receptor 3 ($mGlu_3$) mRNA levels are decreased in brain samples from 36 patients diagnosed with RTT, as well as in mice modeling RTT. $mGlu_3$ is thought to be critically involved in cognition, raising the possibility that decreased in $mGlu_3$ protein levels may contribute to cognitive deficits in RTT patients. We are evaluating the therapeutic potential of $mGlu_3$ in the cognitive deficits associated with RTT. We hypothesize that $mGlu_3$ potentiation using positive allosteric modulators (PAMs) can correct learning and memory impairments, a phenotype seen at the behavioral level in RTT mice, as well as in synaptic plasticity correlates. Future experiments are aimed at determining how loss-of-function mutations in MeCP2 result in decreased $mGlu_3$ expression and substantiating the potential of $mGlu_3$ PAMs to correct abnormal cognition in RTT.

Supported by Pharmacology Training Grant T32 GM07628 (SDV) and a grant from rettsyndrome.org (CMN)

Muscarinic Acetylcholine Receptor 4 (M_4) as a Novel Therapeutic Target for Pitt Hopkins Syndrome

Annah M. Moore, Nicole M. Fisher, Rocco G. Gogliotti, Colleen M. Niswender, J. David Sweatt

Mutations in the gene encoding Transcription Factor 4 (TCF4) cause the neurodevelopmental disorder, Pitt Hopkins Syndrome (PTHS). PTHS is characterized by severe intellectual disability, autism spectrum behaviors, motor incoordination and gastrointestinal issues. There are currently no available treatments for PTHS patients. The Sweatt laboratory has characterized a genetically engineered mouse model of PTHS that shows deficiencies in learning and memory, as well as social deficits that align with the clinical symptoms. Poly(A)+ RNA sequencing studies of wild type (WT) and *Tcf4*$^{+/-}$ mouse hippocampal tissue showed a significant increase in *Chrm4* transcript levels in *Tcf4*$^{+/-}$ mice, the gene that encodes the M_4 muscarinic acetylcholine receptor. Recent work from the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) has shown that potentiation of M_4 receptor signaling yields cognitive enhancing effects in wild-type mice as well as mouse models of psychosis, positioning this receptor as a novel therapeutic target for schizophrenia. Coincidentally, single nucleotide polymorphisms (SNPs) in *TCF4* have been significantly associated with schizophrenia. These connections brought us to ask the question whether M_4 receptor potentiation can also ameliorate the cognitive deficits of PTHS model mice. We report here that a positive allosteric modulator of the M_4 receptor exhibits cognitive enhancing effects in PTHS mice in multiple learning and memory assays. Excitingly, in addition to our results in a rodent model of PTHS, M_4 potentiation has also shown therapeutic effects in models of other neurodevelopmental disorders, such as Rett syndrome and Fragile X syndrome, where it has recently been shown that enhancement of M_4 receptor signaling can normalize cognitive phenotypes. Fragile X syndrome model mice also show a significant increase in hippocampal *Chrm4* transcripts, while Rett syndrome model mice show a significant decrease in hippocampal *Chrm4* transcripts. These data suggest regulation of M_4 signaling in the hippocampus is important for cognitive function in multiple neurodevelopmental disorders. The current study aims to validate potentiation of M_4 receptor signaling as a therapeutic strategy for the treatment of PTHS.

Support

The Pitt Hopkins Syndrome Foundation generously supported this work through a graduate student fellowship.

SF-1 regulation by the acyl chains of phospholipids

Jamal M. Bryant[1] and Raymond D. Blind[2,3]
Departments of [1]Pharmacology, [2]Biochemistry, and [3]Medicine
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Vanderbilt University School of Medicine, Nashville, TN 37232

The nuclear receptor Steroidogenic Factor-1 (SF-1, gene: NR5A1) is a transcription factor that binds DNA controlling the transcription of steroidogenic genes in several endocrine tissues. At the molecular level, SF-1 is known to bind to phospholipids, and we previously demonstrated that the signaling phosphoinositide PI(3,4,5)P3 (PIP3) directly binds and activates SF-1. We also showed that although PI(4,5)P2 (PIP2) binds SF-1, it does not activate SF-1 transcription. SF-1 also has the remarkable *in vitro* ability to shuttle PIP3 between vesicles in an ATP-independent process, a hallmark of all phospholipid transfer proteins. Despite how well we have characterized the PIP3 headgroup regulation of SF-1, how SF-1 is regulated by the chemical nature of the phospholipid acyl chains remains completely uninvestigated in any of these processes.

Here, we will determine the effect of PIP3 acyl-chain length and the degree of saturation on the structure, function, and phospholipid transfer activity of SF-1. In **Aim 1**, we will determine the effect of PIP3 acyl-chain length on a common readout of SF-1 transcriptional activity (recruitment of co-activator peptide to SF-1) using standard biophysical techniques (surface plasmon resonance and microscale thermophoresis). **Aim 2** then uses standard electron paramagnetic resonance (EPR) spectroscopy to quantitate, for the first time, the phospholipid transfer activity of the SF-1 transcription factor. We previously published that SF-1 has phospholipid transfer activity, here we quantitate that activity so SF-1 can be compared to other phospholipid transfer proteins kinetically. **Aim 3** will then use X-ray crystallography to analyze any changes in SF-1 conformation induced by different PIP3 acyl chains. Preliminary data demonstrate that longer PIP3 acyl-chains bind with better affinity to SF-1 than PIP3 species that readily co-crystalize with SF-1. In our highest risk-reward experiments, **Aim 4** will use innovative protein transfection and electroporation to transduce cells with SF-1 protein complexed with a fluorophore-labeled-phospholipid, and determine if SF-1 is capable of phospholipid transfer activity in living cells using microscopy. Altogether, these studies will determine the role of the PIP3 acyl chains in regulating SF-1 transcriptional and phospholipid-transfer activities.

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1:45 pm – 2:30 pm **3MT®-style Presentations 2**
2nd Year Pharmacology Students

Mark Crowder (Blind Lab)
"Characterizing a role for IPMK, a lipid kinase, in liver physiology"

Andrew Feigley (Davies Lab)
"Investigating the possible mechanisms of N-acylethanolamines (NAEs) that alter weigh gain and appetite"

Eric Figueroa (Denton Lab)
"Molecular pharmacology of swelling-activated LRRC8 anion channels"

Breanne Gibson (Schoenecker Lab)
"Targeting the acute phase response to improve tissue repair following traumatic injury"

Chris Hofmann (Emeson Lab)
"Functional consequences of RNA editing on $mGlu_4$ dimerization"

Corey Seacrist (Blind Lab)
"Exploring the Signaling Network of Inositol Polyphosphate Multikinase"

Mabel Seto (Lindsley Lab)
"Effort toward probe development for the metabotropic glutamate receptor type 7 ($mGlu_7$)"

Kayla Shumate (Emeson Lab)
"CAPS1 RNA editing: a modulator of synaptic transmission"

Brittany Spitznagel (Weaver Lab)
"Selective Slack Modulators for the Treatment of Rare Childhood Epilepsy"

Mark Crowder (Blind Lab)

"Characterizing a role for IPMK, a lipid kinase, in liver physiology"

Inositol polyphosphate multikinase (IPMK) is an inositol and PI3-kinase that has been shown to be involved in diverse cellular processes ranging from transcriptional regulation to Akt activation. Notably, its effects can be mediated through both enzyme dependent and independent mechanisms. Since it is a lipid metabolizing enzyme, I am interested in understanding how it affects metabolism in the liver; this will be accomplished using an *IPMK* knockout mouse model. Additionally, due to IPMK's PI3K activity and enzyme-independent Akt activation, I plan to investigate a potential role for IPMK as a driver of liver cancer, the 3rd leading cause of cancer deaths in the United States. These studies aim to characterize new functions for IPMK, an understudied lipid kinase, in the liver and establish basic mechanisms by which operates.

Andrew Feigley (Davies Lab)

"Investigating the possible mechanisms of N-acylethanolamines (NAEs) that alter weigh gain and appetite"

Current treatments for obesity are limited. One potential new strategy to treat obesity is to increase levels of N-acyl-ethanolamides (NAEs). These lipids are synthesized in response to food intake and act to reduce appetite and increase metabolism. Because levels of NAEs are reduced in obese individuals, I am using mouse and zebrafish models to determine the mechanisms that regulate the expression and activity of NAE biosynthetic enzymes and why these enzymes are dysregulated in obesity. I am also investigating the potential of probiotic bacteria engineered to synthesize NAEs as a possible therapeutic approach to increase NAE levels and thereby prevent or treat obesity.

Eric Figueroa (Denton Lab)

"Molecular pharmacology of swelling-activated LRRC8 anion channels"

Cells critically regulate their volume in response to hypoosmotic swelling by transporting chloride and small organic osmolytes out of the cell through so-called volume-regulated anion channels, or VRACs. The molecular identify of this ubiquitous channel was solved after more than two decades in genome-wide siRNA screens for genes that encode VRAC. The channel is likely comprised of up to six subunits encoded by genes LRRC8A-E. LRRC8A is essential for channel activity and likely assembles with other subunits in a cell-type-dependent manner to form volume-regulated channels. Since identifying the gene family, the channel has been implicated in anti-cancer drug uptake, excitatory amino acid efflux, and insulin signaling. Studying the physiology and therapeutic potential of LRRC8 channels would be facilitated by developing small-molecule inhibitors and activators that modulate specific sub-types of LRRC8 channels. Therefore, the **Specific Aims** of my thesis project are: 1) Test the hypothesis that DCPIB and carbenoxolone are pore blockers of LRRC8 channels; 2) Test the hypothesis that DCPIB and carbenoxolone activity are modulated by the subunit composition of LRRC8 channels; 3) Identify novel LRRC8 inhibitors and activators in a small-molecule high-throughput screen.

Breanne Gibson (Schoenecker Lab)

"Targeting the acute phase response to improve tissue repair following traumatic injury"

Following an injury, the body undergoes a systemic response that initially prevents bleeding and infection, and later, it promotes regeneration of injured tissues. In the case of a traumatic injury, the initial phase of this systemic response is exaggerated, resulting in a continuous cycle of inflammation, coagulation, and fibrinolysis. This state of deranged hemostasis not only increases the risk of blood loss, thrombosis, infection, and multi-organ failure, but it also consumes circulating regenerative factors needed for tissue regeneration. Consequently, if trauma patients survive this initial response to their injuries, they often experience poor tissue repair. In order to improve both survival and tissue repair in trauma patients, it is critical that we develop tools to identify and therapeutically target elements of the systemic response to traumatic injury.

Chris Hofmann (Emeson Lab)

"Functional consequences of RNA editing on $mGlu_4$ dimerization"

Group III metabotropic glutamate receptors ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) are members of the G-protein coupled receptor superfamily and have been implicated in numerous neurological disorders including addiction, depression, anxiety, and neuroblastoma. Positive allosteric modulators (PAMs) highly selective for $mGlu_4$ show potent antiparkinsonian effects in rodent models and knockout mice appear resistant to alcohol addiction. The $mGlu_4$ receptor is localized to the presynaptic membrane where it functions as an autoreceptor controlling synaptic release of neurotransmitter. Though initially thought to exist exclusively as a homodimer, recent studies have shown that $mGlu_4$ also can exist as a heterodimer with $mGlu_2$, and that these different dimer pairs are synapse-specific. The sequence of pre-mRNA transcripts encoding $mGlu_4$ can be modified by adenosine-to-inosine (A-to-I) RNA editing events at up to 4 sites to generate as many as 8 receptor isoforms. These editing events result in non-synonymous amino acid alterations in a region of the receptor proposed to represent the interface at which mGlu receptors dimerize. While it is now known that $mGlu_4$/$mGlu_2$ heterodimers exist, it remains to be determined whether RNA editing can affect $mGlu_4$ homo- or heterodimerization.

Using high throughput sequencing, 4 editing sites have been validated within the region of mRNA encoding the ligand binding domain of the $mGlu_4$ receptor and determined the most frequently expressed transcript isoforms. Current studies in the lab focus on determining the extended duplex structures required for editing at each site. Functional studies in this project will involve distinguishing edited receptor isoform stability, signaling, and formation of homo- or heterodimers between these edited receptors and other mGlu receptors.

Corey Seacrist (Blind Lab)

"Exploring the Signaling Network of Inositol Polyphosphate Multikinase"

Inositol polyphosphate multikinase (IPMK) is a non-canonical nuclear PI3K enzyme, which plays critical roles in transcriptional regulation, mTOR nutrient sensing, AMPK regulation and nuclear Akt/PKB signaling. Further, our lab have shown IPMK antagonizes a specific nuclear PTEN activity completely decoupled from all other known PI3K enzymes: IPMK directly phosphorylates $PI(4,5)P_2$ (PIP_2) bound to the nuclear receptor steroidogenic factor-1 (SF-1) activating SF-1 target genes. Although classic p110 PI3K enzymes lack activity on SF-1/PIP_2, PTEN efficiently opposes IPMK action on SF-1. These data demonstrate despite being one of the most heavily targeted enzyme families for cancer therapy, classical PI3K inhibitors cannot compensate for all loss of PTEN functions. Thus, establishing how IPMK has the unique ability to phosphorylate protein/PIP_2 complexes will help elucidate how nuclear PTEN is decoupled from classic p110 PI3K enzymes.

I have determined a 2.9 Å crystal structure of the catalytic core of IPMK, which revealed the classical inositol phosphate kinase fold is conserved in the human IPMK, I have also determined removal of the unstructured regions in IPMK increases its catalytic efficiency ~10-fold compared to the wildtype human IPMK, suggesting the unstructured regions have important biological implications and are not necessary for proper enzymatic function of IPMK.

I am proposing to use a discovery-based approach to find novel protein/PIP_2 substrates of IPMK. In this approach, a "hole" is introduced into the ATP binding fold of the kinase, allowing for utilization of a "bump" ATP, guaranteeing only our mutant IPMK will be able to label substrates with this unnatural ATP. These substrates will be subsequently identified via unbiased mass spectrometry. This technique will also allow me to use "hole" specific kinase inhibitors to determine how short-term inhibition of IPMK's catalytic activity changes cellular physiology. Together, these studies will provide a broad framework of how IPMK functions on the molecular level.

Mabel Seto (Lindsley Lab)

"Effort toward probe development for the metabotropic glutamate receptor type 7 ($mGlu_7$)"

The etiology and treatment of neurological disorders are subjects that have been of much interest for many years. Metabotropic glutamate receptors have been implicated in anxiety, depression, epilepsy, and schizophrenia among others and may emerge as new therapeutic targets. Of particular interest to us is the metabotropic glutamate receptor type 7 ($mGlu_7$), which is widely expressed throughout the brain. Although the function of metabotropic glutamate receptors, including $mGlu_7$, have been previously probed in several disease states, the lack of specific tool compounds has made it difficult to elucidate $mGlu_7$'s exact role(s). The development and optimization of more selective tool compounds for the receptor will help us to further understand the biology of $mGlu_7$ as well as its roles in disease.

Kayla Shumate (Emeson Lab)

"CAPS1 RNA editing: a modulator of synaptic transmission"

The release of peptides and neurotransmitters from neuroendocrine cells and synaptic terminals occurs via regulated exocytosis where secretory vesicles fuse with the plasma membrane in response to a stimulus, typically an increase in free intracellular calcium concentrations. Calcium dependent activator protein for secretion 1 (CAPS1) is a cytosolic protein postulated to act as a priming factor by facilitating or stabilizing the interaction of vesicle- and plasma membrane-associated SNARE proteins. Transcripts encoding CAPS1 are subject to a single adenosine-to-inosine RNA editing event within a region encoding the C-terminal domain (CTD) to convert a genomically-encoded glutamate (GAG) to a glycine (GIG) codon (E/G site). Previous studies have identified the CTD as necessary for localization of CAPS1 to LDCVs and required for Ca^{2+}-triggered LDCV release. Recent work in our laboratory has revealed substantial differences in synaptic vesicle release and/or recycling due to differences in CAPS1 RNA editing. We also have found these pre-synaptic changes to manifest as alterations in post-synaptic signaling, demonstrating the importance of CAPS1 editing in neurotransmission and a potential role in the modulation of synaptic plasticity. Current studies in our lab seek to understand the precise mechanism by which RNA editing impacts CAPS1 protein-protein interactions at SV/LDCV membranes and the physiological role of CAPS1 editing.

Brittany Spitznagel (Weaver Lab)

"Selective Slack Modulators for the Treatment of Rare Childhood Epilepsy"

Malignant migrating partial seizures of infancy (MMPSI) is a rare, severe form of epilepsy that begins in early childhood, with most children experiencing recurrent seizures before six months of age. Due to the severity, and number of seizures, which can range from five to 30 a day, affected individuals can suffer profound developmental delays and intellectual impairment. The majority of MMPSI cases are considered pharmacoresistant causing affected individuals to not survive past infancy or early childhood. At least 20 *de novo*, gain-of-function mutations affecting three functional domains of the potassium channel Slack have been reported in patients with MMPSI, making it the most frequent genetic cause of MMPSI. Slack is a neuronal potassium channel found specifically within inhibitory interneurons. It has been proposed that these mutations cause prolonged hyperpolarization resulting in an imbalance between neuronal excitation and inhibition leading to impaired inhibitory neuronal function and seizures. There is currently an unmet need to identify more selective drugs for Slack associated epilepsies. Discovery and characterization of such compounds may provide a foundation for developing new clinical tools for the treatment of MMPSI.

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2:30 pm – 3:30 pm	**Poster Session**
A-1	Kristopher Abney, MMC **"ACTIVATION OF G PROTEIN-GATED INWARDLY-RECTIFYING POTASSIUM (GIRK) CHANNELS AS A NOVEL THERAPEUTIC TARGET FOR PAIN"**
A-2	Lillian Brady, Postdoctoral Fellow (Hamm Lab) **"The role of complexin on Gβγ-SNARE interaction and the regulation of exocytosis by $G_{i/o}$-coupled G-protein coupled receptors"**
A-3	Claire DelBove (Zhang Lab) "**Gamma-secretase and cholesterol modulates neuronal surface expression of full-length amyloid precursor protein**"
A-4	Yuanjun Guo (Lal Lab) **"Essential Role of HIPK2 in Maintaining Basal Cardiac Homeostasis"**
A-5	Kenneth Harris, MMC (Ramesh Lab) **"SEX-SPECIFIC DIFFERENCES IN BENZO(A)PYRENE [B(a)P]-INDUCED COLON CARCINOGENESIS"**
A-6	Danyeal Heckard, MMC **"Selective activation of membrane estrogen receptors decrease GIRK channel function to rapidly attenuate opioid receptor-like 1 (ORL1) receptor-mediated modulation of nerve injury-induced tactile hypersensitivity"**
A-7	Hussain Jinnah (Emeson Lab) **"RNA Editing-Mediated Regulation of Serotonin 2C Receptor Expression"**
A-8	Krystian Kozek (Weaver Lab) **"Discovery and Development of Tools to Study the Role of G Protein-gated Inwardly-rectifying Potassium (GIRK) Channels in Addiction"**
A-9	Sudan Loganathan, MMC **"Epigenetic-based Combination Therapy for the Treatment of Ewing Sarcoma"**

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A-10	Stephanie Moore (Schoenecker Lab) **"Traumatic Skeletal Muscle Calcification: A Balance of Pyrophosphate and Inorganic Phosphate"**
A-11	Shan Parikh (Knollmann Lab) **"T3+Dex generates functional t-tubules in hiPSC-CM"**
A-12	Kristin Peterson (Hasty Lab) **"Novel regulation of insulin receptor processing & signaling by complement factor 5"**
A-13	Aparna Shekar (Galli Lab) **"A rare, autism-associated in-frame deletion in the dopamine transporter exhibits profound functional deficits"**
A-14	Megan Shuey (Brown Lab) **"Differences in the Treatment of Resistant Hypertension in African Americans and European Americans in a Clinical Setting"**
A-15	Ebrahim Tahaei (Elefteriou Lab) **"The reduced osteogenic differentiation potential of *Nf1*-deficient osteoprogenitors is TGFβ and EGFR-independent"**
A-16	Emily Warren (Konradi Lab) **"Decreased mtDNA decreases mitochondrial creatine kinase: Energetic instability in L-DOPA Induced Dyskinesia"**
A-17	David Wooten and Christian Meyer (Quaranta Lab) **"Quantifying drug synergy along axes of potency and efficacy"**
A-18	YunYoung Yim (Hamm Lab) "G protein specificity of inhibitory adrenergic α_{2a} receptor mediated modulation of synaptic transmission and SNARE"
A-19	Yuantee Zhu (Elefteriou Lab) **"Tissue-specific role of norepinephrine transporter in age-related bone loss"**

Kristopher K. Abney, Michael Bubser, Corey Hopkins, Craig Lindsley, Tom Bridges, Scott Daniels, Carrie Jones, and Dave Weaver

ACTIVATION OF G PROTEIN-GATED INWARDLY-RECTIFYING POTASSIUM (GIRK) CHANNELS AS A NOVEL THERAPEUTIC TARGET FOR PAIN

Meharry Medical College, Department of Neuroscience & Pharmacology, Nashville, TN and Vanderbilt University Department of Pharmacology, Nashville, TN

In the United States approximately 100 million Americans suffer from chronic or acute pain. Symptoms can manifest from a myriad of circumstances including post-operative surgical pain, sports injuries, chemotherapy-induced neuropathy, and diabetic neuropathy. The most common clinically prescribed way to treat pain involves the use of opioid analgesics. However, side effects associated with opioids are detrimental; involving a decrease in quality of life resulting from opioid induced constipation, drug tolerance, and most notably, addiction. Opioid receptors (ORs) are G Protein-Coupled Receptors (GPCR) that signal through $G\alpha_{i/o}$ heterotrimeric G proteins. ORs couple to a variety of downstream effectors that mediate their analgesic efficacy and side effects. G protein-gated inwardly-rectifying potassium (GIRK) channels are one such effector that couple to ORs and produce analgesia. These GIRK channels play an important role in controlling resting membrane potential and cellular excitability. GIRK channels form tetrameric complexes that are comprised of four closely related subunits: GIRK1-GIRK4. Neurons along the main pain pathway, the spinothalamic tract, express GIRK1/2 in the same regions as ORs. Furthermore, OR-mediated activation GIRKs decreases the excitability, which ultimately inhibits excitatory activities in both afferent neurons and second order neurons that carry noxious stimuli to higher cortical areas of the brain. GIRK's contribution to nociception is demonstrated in GIRK1 and GIRK2 knock-out mice, where both mice show attenuated responses to morphine, an OR agonist. Recently, our lab developed a series of potent and selective modulators of GIRK1-containing channels. Previous studies have shown *in vivo* administration of some of these novel compounds decreases seizure-like activity in murine models of epilepsy, and also reduces anxiety-like behavior in murine models of anxiety. We hypothesize that further development of potent and selective GIRK1/2 activators may provide analgesia through a novel mechanism and that GIRK-induced analgesia may be free from the side effects associated with opioids.

The role of complexin on Gβγ-SNARE interaction and the regulation of exocytosis by $G_{i/o}$-coupled G-protein coupled receptors

Lillian J. Brady, PhD, Zack Zurawski, PhD, Karren Hyde, Qi Zhang, PhD, and Heidi E. Hamm, PhD

A variety of neurological diseases are treated by targeting presynaptic $G_{i/o}$-coupled G-protein coupled receptors (GPCRs). The modulation of the activity of these types of receptors leads to presynaptic regulation of fast neurotransmitter release, an important concept underlying synaptic transmission. There are several types of fast synaptic transmission, including spontaneous and evoked synchronous release of neurotransmitters from presynaptic terminals, requiring the SNARE complex and associated exocytotic machinery proteins. The βγ subunit of specific $G_{i/o}$-coupled GPCRs has been shown to not only modulate voltage-dependent Ca^{2+} channels (VDCC) and activate G-protein inward rectifying potassium (GIRK) channels, but also work downstream of Ca^{2+} entry by binding to the SNARE complex and directly competing with the calcium sensor Synaptotagmin I (Syt1) on tSNARE. This mechanism inhibits evoked synchronous release and modifies spontaneous release that is independent of VDCC and GIRK channel-meditated modulation.

A central component to vesicle fusion during the process of spontaneous and evoked neurotransmitter release is the indirect or direct binding of different exocytotic machinery proteins to the SNARE complex as well as individual SNARE proteins. One such protein is complexin, which is thought to act as a clamp to synchronize vesicle fusion. Complexin binds concurrently with synaptotagmin to the SNARE complex in the physiological state; however it is not known whether Gβγ can bind to SNARE in this state. The purpose of this investigation is to use biochemistry techniques to determine whether the Gβγ-SNARE interaction will be in competition or coordination with complexin. Preliminary results show that complexin reduces the rate but not the maximum extent of Syt1-driven lipid mixing in the absence of Gβγ, and that Gβγ is still able to inhibit Syt1-driven lipid mixing in the presence of complexin. These results support the hypotheses that Gβγ and complexin can bind noncompetitively to the SNARE complex and that Gβγ does not interact with complexin.

Future studies will utilize whole-cell patch clamp electrophysiology on primary rat hippocampal cultures to investigate whether $G_{i/o}$-coupled GPCR-mediated inhibition of exocytosis is dependent upon or independent of complexin, in addition to its dependence on the interaction of Gβγ with SNARE. Previously it has been shown that the 5-$HT_{1β}$ and $GABA_B$ $G_{i/o}$-coupled receptors function via the Gβγ-SNARE interaction to inhibit spontaneous and evoked release. Using agonists of these receptors to modulate their activity, it will be determined whether these receptors can still inhibit neurotransmitter release in complexin-containing and complexin-deficient synapses. Overall, this study will provide mechanistic insight into the Gβγ–SNARE interaction and provide a foundation for future studies leading to the development of better pharmaceutical agents used to treat a variety of neurological diseases.

Gamma-secretase and cholesterol modulates neuronal surface expression of full-length amyloid precursor protein

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Amyloid precursor protein (APP) is a ubiquitously expressed neuronal membrane protein with a single transmembrane domain. It is sequentially processed by three secretases to produce beta-amyloid peptides, the major component of amyloid plaque. Inhibition of the amyloidogenic gamma-secretase (gS) has been one of the major therapeutic strategies for Alzheimer's disease. Unfortunately, little success has been made so far. It is not well understood how gS inhibition alters the fate of full-length APP, which may cause unexpected consequences. In addition, gS is also correlated to cholesterol, a multifaceted lipid enriched in neuronal membrane. To directly explore these questions in live neurons, we conducted live-cell fluorescence imaging in conjunction with a set of biochemical measurements. We constructed a multicolor pH-sensitive fluorescent APP as a ratiometric reporter for its trafficking and proteolytic processing. Using immunocytochemistry, we confirmed that the expression and localization of the fluorescent APP resembles endogenous APP. We also confirmed that its trafficking and processing were consistent with that of endogenous APP. In combination with a functional synaptic label, we also examined the relationship between synaptic activity and APP trafficking and processing. We found that APP enrichment at synaptic boutons was proportional to synaptic membrane abundance and that APP trafficking or processing was loosely correlated to synaptic activity. Upon gS inhibition, we unexpectedly observed significant surface accumulation of full-length APP along with the increase of its C-terminal fragments. In comparison to the synaptic vesicular membrane label, we discovered that this accumulation was correlated to the surfacing of synaptic vesicles. Intriguingly, the observed effects of gS-inhibition could be replicated by the depletion of membrane cholesterol, consistent with the idea that cholesterol, enriched in synaptic membranes, allosterically associates with gS and contributes to the amyloidogenic proteolysis of APP. This is further suggested by the evidence for interplay between gS and other secretases. In summary, our study demonstrates that gS and cholesterol collaboratively regulate the trafficking and processing of APP, which implicates the role of APP and secretases in the homeostasis of neuronal membrane cholesterol and thus a plethora of cholesterol-associated neuronal functionalities.

Essential Role of HIPK2 in Maintaining Basal Cardiac Homeostasis

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Heart failure is the leading cause of mortality and morbidity worldwide. Despite progresses made in the treatment of most cardiovascular diseases, the prevalence and mortality of heart failure are still rising. Thus, new targets for heart failure treatment are in desperate need. Based on an integrated transcriptional analysis, we identified a previously unknown target—Homeodomain-interacting protein kinase 2 (HIPK2), potentially involved in cardiac remodeling process. HIPK2 is a conserved nuclear serine-threonine kinase with a well-established role in cancer biology. However, the role of HIPK2 in cardiac biology is unknown. To determine the role of HIPK2 in the heart, we generated αMHC-Cre driven cardiomyocyte-specific HIPK2 knockout (KO) mice. We found that the heterozygous HIPK2 KO mice (Het, $HIPK2^{flox/\ \alpha MHC\text{-}Cre/}$) developed cardiac dysfunction at the age of 6 months, as reflected by significantly reduced fractional shortening and ejection fraction, while heart function was comparable between the WT and Het mice at 3 months of age. Consistent with the decreased contractile function, the left ventricle internal dimension at end-diastole and systole was also enlarged in the Het mice. In the *in vitro* study using neonatal rat ventricular cardiomyocytes, overexpression of HIPK2 suppressed the expression of *Nppa* and *Nppb* at basal condition. These findings suggest that cardiomyocyte HIPK2 is required to maintain normal cardiac homeostasis and its deletion leads to marked cardiac dysfunction. Overexpression of HIPK2 is protective for the cardiomyocyte from pathological hypertrophy.

SEX-SPECIFIC DIFFERENCES IN BENZO(A)PYRENE [B(a)P]-INDUCED COLON CARCINOGENESIS

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Colorectal cancer (CRC) is the third most common diagnosed cancer and the third leading cause of cancer-related deaths in the United States. It has also been reported that colon cancer incidence and mortality rates are higher in men than woman, but there is yet a determined mechanistic link to show the factors that underlie the sex-specific differences in CRC initiation and progression. Benzo(a)pyrene [B(a)P], a member of the polycyclic aromatic hydrocarbon (PAH) family of compounds is a well-characterized environmental toxicant that has been proven to be a major contributor to the development of sporadic colon cancer. Published studies indicate that Aryl Hydrocarbon Receptor (AhR), a receptor for [B(a)P], bind to estrogen receptor (ER) and negatively affect AhR-target gene transcription. This study aims to elucidate the sex-specific differences in B(a)P-induced colon cancer in adult Polyposis In the Rat Colon (PIRC) model. We hypothesize that sex-specific differences in B(a)P biotransformation modulates the formation of colon tumors in PIRC rats. Groups of female and male PIRC rats (n = 8) received sub-chronic exposure to 25, 50 and 100 µg B(a)P/kg body wt. via oral gavage for 60 days. Female and male rats that received no [B(a)P] treatment served as controls. [B(a)P] was shown to have no significant effect on body weight of these rats and female PIRC rats that received 25, 50 and 100 µg B(a)P/kg body wt. showed significant decrease in total polyp count when compared to males with respective treatments. Polyp sizes of female PIRC rats receiving 25, 50 and 100 µg B(a)P/kg body wt. were increased when compared to males respectively. Histopathological analysis of colon polyps revealed that female animals exhibited low-grade to no dysplasia while high-grade dysplasia was recorded in male animals treated with corresponding doses. In future studies, by measuring the expression of phase 1 and phase 2 drug metabolizing enzymes (DME), along with measuring circulating estrogen levels, analyzing [B(a)P] metabolite profile, and probing B(a)P-DNA interactions, we will provide insight into if and how estrogen receptor protects females from developing colon cancer. This research was funded by NIH grants 5RO1CA142845-04, 5R25GM059994-3, and G12MD007586-29.

Selective activation of membrane estrogen receptors decrease GIRK channel function to rapidly attenuate opioid receptor-like 1 (ORL1) receptor-mediated modulation of nerve injury-induced tactile hypersensitivity

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Numerous studies have reported that women have a higher prevalence of chronic pain disorders than men. We have previously shown that estrogen attenuates opioid receptor like -1 (ORL1) receptor mediated thermal antinociception in females [Claiborne et al. J Neurosci. 26:13048-53, 2006]; and down regulates the ORL1 gene expression [Flores et al. Neurosci. 118:769-78, 2003]. Recently, we have demonstrated that activation of membrane estrogen receptors (GPR30, Gq-mer, ER α, but not ER β) abolishes ORL1-mediated acute thermal antinociception via an ERK2-dependent non-genomic mechanism [Small et al. Neurosci. 255:177-190, 2013]. However, the role of membrane estrogen receptors (mERs) in modulating ORL1-mediated attenuation of neuropathic pain as well as the molecular mechanisms involved remain unknown. Thus, the present study investigated whether activation of mERs attenuates ORL1-mediated modulation of nerve injury-induced tactile hypersensitivity. The thallium flux assay was utilized to determine the effect of mER activation on GIRK channel function. The spared nerve injury (SNI) model as previously described by Decosterd and Woolf [Pain. 87:149-58, 2000] was employed to induce mechanical hypersensitivity in male and OVX female Sprague Dawley rats. After a 7-day recovery period, sham and SNI rats were intrathecally administered a selective mER agonist immediately followed by OFQ, the endogenous ligand for the ORL1 receptor, into the lumbosacral spinal cord of rats through an implanted PE-10 cannula. Paw withdrawal thresholds (PWTs) were recorded using an automated dynamic plantar aesthesiometer. SNI significantly reduced PWTs in both males and OVX females. Intrathecal administration of OFQ significantly increased PWTs in both males and OVX females while selective mER activation abolished OFQ-induced increase in PWTs. Thus, we conclude that activation of mERs rapidly attenuates ORL1-mediated modulation of nerve injury-induced tactile hypersensitivity by dampening GIRK channel function. This work provides evidence of a biological mechanism that increases female vulnerability to the development of chronic pain disorders.

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RNA Editing-Mediated Regulation of Serotonin 2C Receptor Expression

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Pre-mRNA transcripts encoding the 2C subtype of the serotonin receptor ($5HT_{2C}$) can be differentially edited by RNA-specific adenosine deaminases at five closely spaced positions within exon 5. These A-to-I editing events can generate as many as 24 protein isoforms that differ by up to three amino acids within the predicted second intracellular loop of the receptor, a region essential for G protein coupling. Functionally, the highly edited $5HT_{2C}$ isoforms (e.g. $5HT_{2C\text{-}VSV}$ and $5HT_{2C\text{-}VGV}$) exhibit reduced constitutive activity and altered subcellular localization in comparison to the genomically-encoded isoform ($5HT_{2C\text{-}INI}$). Furthermore, alterations in $5HT_{2C}$ editing have been observed in patients diagnosed with schizophrenia, in suicide victims with a history of major depression, and in response to antidepressant and antipsychotic treatments, suggesting that improper editing of $5HT_{2C}$ transcripts may be a contributing factor in neuropsychiatric illness. Recent work in our lab has shown that genetically modified mice solely expressing the fully edited $5HT_{2C}$ receptor isoform ($5HT_{2C\text{-}VGV}$) produce an anomalous 40- to 70-fold increase in receptor density without a concurrent change in steady-state $5HT_{2C}$ mRNA, indicating that the relative expression of $5HT_{2C}$ protein isoforms is not represented by the steady-state level of edited $5HT_{2C}$ mRNAs. The molecular mechanism(s) underlying this novel disparity between mRNA and protein isoform expression have yet to be elucidated. To confirm whether such a disparity exists between edited $5HT_{2C}$ transcripts and their encoded protein products in wild-type mice, I am using affinity purification methods to isolate $5HT_{2C}$ receptors, followed by a mass spectrometry-based proteomic analysis to quantify the relative expression levels of $5HT_{2C}$ protein isoforms. Due to the low expression of this G-protein coupled receptor in most regions of the mouse brain, I employed a CRISPR/Cas9-based approach to generate knock-in mice in which hexahistidine- and *Strep-II*® affinity tags have been "knocked-in" to the endogenous $5HT_{2C}$ locus. Deep sequencing has confirmed that insertion of the twin epitope tag does not alter normal patterns of RNA editing and slicing of $5HT_{2C}$ transcripts. Purification efficacy has also been validated in a heterologous system (HEK 293) expressing this affinity-tagged 5-HT_{2C} receptor, and subsequent mass spectrometric analysis has detected the desired chymotryptic peptide of interest. Current work is focused on isolating sufficient quantities of the receptor from whole brain and dissected regions for quantitative analysis. Identification of disparities between $5HT_{2C}$ RNA and protein isoform expression will have important implications for human studies of disease-related alterations in $5HT_{2C}$ RNA editing, in which inferences about receptor isoform expression and function have been based solely upon edited mRNA distribution profiles. These findings will also imply the existence of a novel post-transcriptional mechanism for regulating $5HT_{2C}$ receptor expression that may be mediated by differences in translation efficiency and/or protein stability among distinct edited isoforms. Subsequent work will focus on identifying the mechanistic basis underlying this disparity between RNA and protein expression.

Discovery and Development of Tools to Study the Role of G Protein-gated Inwardly-rectifying Potassium (GIRK) Channels in Addiction

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Purpose: Prescription opioid abuse is a nationwide epidemic. Opioids induce a rewarding release of dopamine from dopaminergic (DA) neurons in the ventral tegmental area (VTA) of the brain. These neurons express G protein-gated inwardly-rectifying potassium channels (GIRKs), and, while GIRK channels are composed of GIRK1, 2, 3, and/or 4 subunits that assemble into homo- and heterotetramers, the VTA DA neurons express only non-GIRK1-containing GIRK channels. These GIRK channels are implicated in the regulation of substance abuse. To further investigate this role of GIRKs, we sought to determine if pharmacological modulation of GIRKs in the VTA can affect drug-seeking behavior. However, non-GIRK1-subunit-containing selective GIRK channel modulators do not exist, and I aim to discover and characterize such compounds.

Methods: We screened a library of >110,000 compounds using GIRK2-overexpressing HEK293 cells in a high-throughput thallium-flux assay. Active compounds discovered from the screen were further characterized using a variety of GIRK channel overexpressing cell lines to determine channel specificity using variations of the thallium-flux assay.

Results: From among the most active compounds of the screen, we discovered that the natural product ivermectin activated GIRK2 channels. We found that ivermectin also activated GIRK1/2 and GIRK1/4 channels. The potency of ivermectin on these channels was >10 μM, and the maximum efficacy on GIRK2 channels was the greatest observed to date in thallium flux.

Conclusion: While not exhibiting the desired non-GIRK1-containing channel specificity, ivermectin will enable the pharmacological probing of DA neurons in in vitro experiments. Future studies will explore analogs of ivermectin in search of compounds that are increasingly potent, efficacious, and selectively activate non-GIRK1-containing channels. Further, active compounds will be studied using *in vitro* and *ex vivo* electrophysiology, and we will assess the efficacy of non-GIRK1-containing channel selective compounds in rodent behavioral models of craving and addiction.

Epigenetic-based Combination Therapy for the Treatment of Ewing Sarcoma

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Ewing's sarcoma is a class of highly malignant tumors of the bone and soft tissue commonly afflicting the pediatric and young adult population. There is a strong understanding that the vast majority of this class of sarcomas are driven by the aberrantly activated recurrent chromosomal translocation t(11;22)q(24;12), also termed EWS-FLI1. However, pharmacological intervention targeting EWS-FLI1 oncodriver has been futile. Additionally, Ewing's sarcoma is highly dependent on IGF1 activation for growth and proliferation, making this ailment highly resistant to currently available treatment. In the present study, we demonstrated that BET bromodomain proteins, a group of epigenetic regulators, critically regulate Ewing's sarcoma pathogenesis via down-regulating EWS-FLI1 driven-transcriptional activity. Pharmacological or genetic targeting of BET proteins impaired proliferation and survival in a wide variety of Ewing's sarcoma cells. Moreover, inhibition of BET proteins significantly decreased IGF1 activation of the AKT pathway in Ewing's sarcoma cells. BET bromodomain inhibitors alone suppressed tumor growth and significantly induced regression with combination therapy for Ewing sarcoma xenograft models. BET bromodomain inhibitors alone have shown strong potency due to their multimodal activity of blocking key drivers of Ewing's sarcoma pathogenesis. Our findings suggest a novel therapeutic strategy for treating Ewing's sarcoma by targeting epigenetic regulators that drives sarcoma disease pathogenesis.

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Traumatic Skeletal Muscle Calcification: A Balance of Pyrophosphate and Inorganic Phosphate

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Introduction: Trauma-induced skeletal muscle calcification is a spectrum of disease, including dystrophic calcification and heterotopic ossification (HO), that may occur following severe injuries such as burn, blast, neurologic and musculoskeletal injuries, as well as certain orthopaedic procedures. Under normal physiologic circumstances, when the body undergoes injury, innate protection mechanisms inhibit the formation of calcification within skeletal muscle. Pyrophosphate is a well described anti-mineralization molecule that has been demonstrated to protect skin, the heart, and the kidneys from aberrant mineralization. Through this work, we will investigate the role of pyrophosphate plays in protecting skeletal muscle from mineralization following injury with the hypothesis that the balance of pyrophosphate to inorganic phosphate is critical for limiting mineralization in soft tissues.

Results: Using our validated model of trauma-induced skeletal muscle calcification in combination with either 1) a pyrophosphate deficiency or 2) a high phosphate diet, we demonstrated that the balance of pyrophosphate to inorganic phosphate is critical such that either a loss of pyrophosphate or an enhancement of inorganic phosphate through diet is sufficient to form skeletal muscle calcification following injury. Next, given our findings, we aimed to test our previously successful therapeutic method of enhancing plasmin activity to prevent the initial formation of skeletal muscle calcification in both mouse models. Starting at the time of weening, male mice were fed a high phosphate diet and were then administered an antisense oligonucleotide (ASO) specific for α2-antiplasmin or a non-targeting control (100mg/kg/week) beginning two weeks prior to injury and continuing weekly. Following α2-antiplasmin ASO treatment, we observed a marked decrease the development of skeletal muscle calcification in both models following injury at 7 days following injury as compared to control treated animals ($P=<0.001$). Therefore, these results distinguish plasmin activity as a promising therapeutic target for mitigating the development of skeletal muscle calcification following injury in a plasmin deficiency-independent model.

Discussion: Through this work, we have demonstrated that skeletal muscle calcification following injury is multifactorial. As such, alteration to multiple pathways, either genetic or environmental, can predispose skeletal muscle to calcification. Conversely, we have demonstrated that enhancement of just the fibrinolytic pathway, by targeting plasmin activity, is sufficient to provide cross-protection against high phosphate diet-induced skeletal muscle calcification following injury.

Significance: Since plasmin also supports bone homeostasis and fracture repair, increasing plasmin activity represents the first pharmacologic strategy to prevent skeletal muscle calcification without adversely affecting systemic bone physiology or concurrent skeletal muscle and bone regeneration. Therefore, these findings support further therapeutic development and potential bench to bedside translation.

Title: T3+Dex generates functional t-tubules in hiPSC-CM

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Abstract

Rationale: Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) are increasingly being used for modeling heart disease and are under development for regeneration of the injured heart. However, incomplete structural and functional maturation of hiPSC-CM including lack of t-tubules, immature excitation-contraction (EC) coupling, and inefficient Ca-induced Ca release (CICR) remain major limitations.

Objective: Thyroid and glucocorticoid hormones are critical for heart maturation. We *hypothesized* that their addition to standard protocols would promote t-tubule development and mature EC coupling of hiPSC-CM when cultured on extracellular matrix with physiological stiffness (Matrigel mattress).

Methods and Results: HiPSC-CM were generated using a standard chemical differentiation method supplemented with triiodo-L-thyronine (T3) and/or dexamethasone (Dex) during days 16-30 followed by maturation for 5 days on Matrigel mattress. HiPSC-CM treated with T3+Dex, but not with either T3 or Dex alone, developed an extensive t-tubule network. Notably, Matrigel mattress was necessary for t-tubule formation. Compared to adult human ventricular CM, t-tubules in T3+Dex-treated hiPSC-CM were less organized and had more longitudinal elements. Confocal line scans demonstrated spatially and temporally uniform Ca release that is characteristic of ventricular-like EC coupling. T3+Dex enhanced elementary Ca release measured by Ca sparks as well as promoted ryanodine receptor (RyR2) structural organization. Simultaneous measurements of L-type Ca current and intracellular Ca release confirmed enhanced functional coupling between L-type Ca channels and RyR2 in T3+Dex cells.

Conclusions: Our results suggest a permissive role of combined thyroid and glucocorticoid hormones during the cardiac differentiation process which, when coupled with further maturation on Matrigel mattress, is sufficient for t-tubule development, enhanced CICR, and more ventricular-like EC coupling. This new hormone maturation method could advance the utility of hiPSC-CM for disease modeling and cell-based therapy.

Novel regulation of insulin receptor processing & signaling by complement factor 5

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The complement system is an evolutionarily conserved innate immune signaling pathway that is involved in many responses to infection, but is less recognized for its role in metabolic signaling. Our studies suggest that terminal, and otherwise “inactive”, components of the complement system impact metabolism by altering insulin receptor (InsR) expression and processing– a completely novel link between immunity and metabolism. Given the chemoattractant potential of complement factor 5 (C5), and upregulation of C5 expression in adipose tissue (AT) of high fat fed mice, we originally hypothesized that C5 deficiency would result in reduced inflammation and improved insulin sensitivity. When mice with systemic C5 deficiency ($C5_{def}$) were placed on low fat and high fat diets, the $C5_{def}$ mice gained less weight than their congenic controls, and had reduced liver mass and triglyceride content on the high fat diet, supporting our hypothesis. However, the $C5_{def}$ mice demonstrated severe glucose intolerance and systemic IR, as well as impaired insulin signaling in liver and AT. Adenoviral expression of C5 partially reversed this phenotype. As a novel mechanism affecting insulin signaling, the $C5_{def}$ mice also exhibit improper processing of pro-InsR, and decreased InsR-β gene expression and protein levels. Additionally, we have preliminary data indicating a strong predictive association between reduced C5 expression and T2D in humans. Using PrediXcan, an advanced computational system that can predict associations between disease states and gene network expression levels, we found that genetic variants predictive of lower C5 expression in blood are strongly associated with increased incidence of T2D ($p<0.004$). Overall our data show a novel connection between complement signaling and insulin action via a receptor-level processing change of the InsR.

A rare, autism-associated in-frame deletion in the dopamine transporter exhibits profound functional deficits

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The neurotransmitter dopamine (DA) mediates fundamental behaviors such as reward, motivation, attention, and cognition. Important to dopamine neurotransmission is the dopamine transporter (DAT), a member of the solute carrier family 6 encoded by the *SLC6A3* gene. DAT is a presynaptic membrane protein responsible for the reuptake and recycling of DA following calcium-mediated vesicular release. DAT dysfunction has been associated with neuropsychiatric disorders including ADHD, schizophrenia, bipolar disorder, and autism spectrum disorders (ASD). DAT is also the target of commonly abused psychostimulants and controlled substances, namely cocaine and amphetamine (AMPH).

Recently, our laboratory identified a novel, *SLC6A3* variant identified in an ASD proband from whole exome sequencing. The genetic variant is an in-frame deletion of three nucleotides resulting in a deletion of amino acid Asn336 (ΔN336). Located in the third intracellular loop, N336 is conserved from human to *Drosophila*, and *in silico* algorithms predict that ΔN336 confers a functionally damaging effect to DAT. Expression of human DAT (hDAT) ΔN336 in Chinese Hamster Ovary (CHO) cells revealed a near absence of DAT-dependent DA uptake relative to wildtype DAT, yet surface expression was unchanged. This *SLC6A3* variant is permissible to AMPH-induced efflux, but exhibits diminished DAT-mediated inward currents promoted by rapid application of AMPH. To understand the basis of the ΔN336 variant's effects on transporter conformational dynamics, we conducted electron paramagnetic resonance (EPR) spectroscopy analyses by introducing the correspondent deletion in the leucine transporter, LeuT. LeuT ΔV269 promotes a closed conformation of the extracellular gate and facilitates the intracellular gate to reside in a "half-open and inward-facing" conformation.

The results of this study provide new structural and functional insights into the role of DAT structural domains controlling DA transport. In addition, they define how disruption of specific DAT structural interactions and gate conformations translate to deficits in molecular function. Most importantly, this work adds to the growing body of literature implicating altered regulation of DA homeostasis/transport as a potential biological mechanism underlying liability to ASD.

Differences in the Treatment of Resistant Hypertension in African Americans and European Americans in a Clinical Setting

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The identification of hypertensive patient populations using electronic health records (EHR) has the potential to elucidate novel trends in the real world treatment of hypertension. Using Vanderbilt University Medical Center's synthetic derivative, a de-identified mirror of the EHR, **we tested the hypotheses that we could develop algorithms to identify patients from a clinical population that had resistant hypertension (RH) and identify patterns of treatment within the resistant hypertension population**.

We developed algorithms to identify clinical patients with RH, defined as patients with blood pressure $\geq$ 140/90 mmHg despite concurrent treatment of three antihypertensive classes or patients treated with $\geq$ four classes both must include a thiazide diuretic, amlodipine, or other dihydropyridine calcium channel blocker. The algorithms had positive predictive values, negative predictive values, sensitivities, and specificities that exceeded 92%. From a total hypertensive population containing 186,015 European Americans (EA) and 33,576 African Americans (AA) we identified 13,541 (7.3%) and 3,541 (10.5%) patients with RH, respectively. These prevalence estimates are consistent with previous studies as was the observation that AA patients with RH were younger, heavier, predominately female, and had higher incidence of type II diabetes mellitus than EA.

By comparing the records of RH patients treated with three antihypertensive classes to patients prescribed $\geq$ four classes we were able to elucidate trends in the prescription of add-on antihypertensive therapies. In general, all medication classes, except for thiazide diuretics, were prescribed more frequently in patients treated by $\geq$ four classes than patients treated with three. In particular, initial spironolactone use significantly increased from 2.6% to 12.4% in EA and 2.8% and 12.3% in AA and the prevalence of spironolactone addition to a RH patient's medication record at any point after RH diagnosis increased to 36.6% and 40.3%, respectively. We also identified racial trends in class use including the observation that AA were significantly less likely to be prescribed angiotensin receptor blockers and renin inhibitors and more likely to be prescribed direct acting vasodilators than EA.

The reduced osteogenic differentiation potential of *Nf1*-deficient osteoprogenitors is TGFβ and EGFR-independent

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Neurofibromatosis type 1 (NF1) is a common genetic disorder caused by mutations in the *NF1* gene. It affects multiple organs, including the skeleton. Recalcitrant bone healing following fracture (pseudarthrosis, PA) is one of the most problematic pediatric skeletal complications associated with NF1. The etiology of this condition is still unclear thus pharmacological options to manage it clinically are limited to off-label use of BMPs. Multiple studies have shown that *Nf1*-deficient osteoprogenitors are characterized by a reduced response to osteogenic cues. A recent RNAseq analysis comparing bone cells prepared from the pseudarthrotic site of children with NF1 PA (shown to harbor somatic double hit mutations in *NF1*) versus control iliac crest-derived cells (haploinsufficient for *NF1* mutations) revealed that *EREG* and *EGFR1,* encoding epiregulin and its receptor Epidermal Growth Factor Receptor 1, respectively, were the top over-represented genes in NF1 PA cell cultures. Because EGFR stimulation was shown to inhibit osteogenic differentiation, we hypothesized that chronic EGFR stimulation in *NF1*-deficient skeletal progenitors contributed to their reduced osteogenic differentiation potential. In this study, we confirm first by single cell sequencing that *NF1* second hit somatic mutations in human bone cells is associated with an increase in *EREG* and *EGFR* expression, whereas it does not affect *TGFβ1* expression. We then show that this molecular signature is conserved in mouse bone marrow stromal cells deficient for *Nf1*; However, blocking EGFR signaling by Piozotinib or AG-1478, or the EGFR ligand epiregulin, did not correct the differentiation defect of *Nf1*-deficient bone marrow stromal cells. In addition, we show in a co-culture experimental setting that *Nf1*-deficient BMSCs inhibit the differentiation of *Nf1*+/- BMSCs and their osteogenic response to the EGFR inhibitor Piozotinib. From a translational point of view, these results suggest that available pharmacological strategies aimed at inhibiting EGFR signaling are unlikely to ameliorate the behavior of osteoprogenitors characterized by *NF1* loss-of-function, and emphasize the need for more efforts to identify the mechanism whereby *Nf1* loss-of-function inhibits osteoblast differentiation.

Decreased mtDNA decreases mitochondrial creatine kinase: Energetic instability in L-DOPA Induced Dyskinesia

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L-DOPA Induced Dyskinesia (LID), characterized by excessive and uncontrolled movements, occurs in most Parkinson's Disease patients treated with L-DOPA, but some patients develop LID more quickly than others. One of the factors differentiating dyskinetic PD patients from nondyskinetic patients is a reduction of mtDNA in the striatum. We hypothesize that this decreased mtDNA in the putamen of dyskinetic patients plays a critical role in accelerating the development of LID by destabilizing the striatum's energetic capacity. We have established a model of this mtDNA reduction by treating primary striatal cultures with ethidium bromide (EtBr), which at low concentrations accumulates in mitochondria and selectively reduces mtDNA while sparing nuclear DNA. EtBr-depleted mtDNA induces a dramatic reduction in mtRNA, which corresponds to a decrease in mitochondrial respiratory activity. Moreover, decreased mtDNA in these cultures results in a neuron-specific reduction in the expression of mitochondrial creatine kinase (mtCK), an enzyme crucial for shuttling phosphate from mitochondrially-produced ATP to sites of high energy demand. Expression of mtCK was also significantly reduced in dyskinetic PD patients. This reduction may point to a relationship between mtDNA quantity and an ability to appropriately respond to high energy stimuli.

Title: Quantifying drug synergy along axes of potency and efficacy

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Recent studies have demonstrated biases in the prevailing methods for quantifying drug response in in vitro cell-proliferation assays. We recently reported a new metric for quantifying a single drug's effect which addresses these biases, termed the "drug-induced proliferation (DIP) rate". However, in cancer a single drug is often insufficient to achieve net tumor regression (negative DIP rate) at clinically tolerable doses. Therefore, there is a strong interest in identifying combinations of drugs which cooperate to produce a greater effect than either drug alone, a phenomenon termed drug synergy. Because current methods for quantifying drug synergy are inapplicable to the DIP metric, we developed a new index of drug synergy inspired by a ternary complex model of allosteric interaction. One advantage of our metric over previous metrics is it distinguishes two types of synergy: namely synergistic efficacy and synergistic potency. Importantly, these match the axes of clinical interest: tolerable dose ranges and high efficacy. To demonstrate the potential for our metric in drug combination discovery, we analyze a high through-put screen of drugs in combination with osimertinib (a third generation EGFR-TKI) in PC9 cells (NSCLC). We find the vinca alkyloids vindesine and vinorelbine are synergistically efficacious with osimertinib resulting in complete ablation of residual populations characteristic of either drug in isolation. We also find dasatinib (a SFK inhibitor) to be synergisticly potent with osimertinib suggesting lower doses are necessary for equal efficacy, which may ameliorate off target toxicities in the bone and immune system characteristic of SFK inhibitors.

G protein specificity of inhibitory adrenergic α_{2a} receptor mediated modulation of synaptic transmission and SNARE

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Modulation of neurotransmitter exocytosis by activated Gi/o-type G-protein coupled receptors (GPCRs) is a universal regulatory mechanism used both to avoid overstimulation and to influence circuitry. One of the known modulation mechanisms is Gβγ and soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex interaction. There are 5 Gβ and 12 Gγ subunits, but specific Gβγs activated by a given GPCR *in vivo* are not known. Presynaptic α_{2a}-adrenergic receptors (α_{2a}-ARs) in both adrenergic (auto α_{2a}-ARs) and non-adrenergic neurons (hetero α_{2a}-ARs) inhibit neurotransmitter release and affect various physiological function such as anesthetic sparing and working memory enhancement. Here, we investigate whether auto α_{2a}-ARs in sympathetic neurons use the same Gβγ subunits as hetero α_{2a}-ARs in other neuronal types to inhibit exocytosis by interacting with SNARE. Using several mice models including transgenic Flag-α_{2a}-ARs, knock-in HA-α_{2a}-ARs, co-immunoprecipitation, mass spectrometry analysis, we have determined the Gβ and Gγ subunits that interact with α_{2a}-ARs and SNARE complexes. $G\beta_2$ and $G\beta_4$ preferentially interact with activated α_{2a}-ARs while only $G\beta_2$ is selective for auto-α_{2a}-ARs. We also see a basal Gβγ-SNARE interaction and the 2 fold enhancement of this interaction upon the auto α_{2a}-ARs activation. Further understanding Gβγ specificity and Gβγ-SNARE interaction may offer new insights into the normal functioning of the brain, as well as better understanding of disease progression.

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Tissue-specific role of norepinephrine transporter in age-related bone loss

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Osteoporosis is a bone metabolic disease affecting approximately 14 million Americans over the age of 50, resulting in increased risk of fractures and associated morbidities. The prevalence of the disease increases with age, independent of hormonal status (e.g. menopause). Understanding the pathology and developing clinical interventions for age-related osteoporosis depends on understanding bone remodeling – a process regulated by local and systemic signals – and how these signals are affected during aging. The sympathetic nervous (SNS) system controls bone remodeling via norepinephrine release, which directly inhibits bone formation by osteoblasts and promotes bone resorption by osteoclasts. Mice with global genetic deletion and chronic pharmacologic blockade of the norepinephrine transporter (NET) have decreased bone mass, suggesting NET is important for acquisition of peak bone mass. NET is expressed and functional in osteoblasts as well as neurons, i.e. takes up extracellular norepinephrine, and thus putatively down-regulates the effects of the SNS on local tissues such as bone. Aged 18 month old mice also have high basal SNS tone, and we postulate that this is partially etiologic to age-related bone loss in these animals. The proposed project aims to determine whether NET in osteoblasts or neurons modulate bone remodeling, and whether a reduction in NE uptake by bone cells during aging contributes to bone loss. This will be accomplished by analyzing the bone phenotypes at different ages of mice with floxed *Net* allele tissue-specific deletions, using the sympathetic neuron-specific Tyrosine Hydroxylase-Cre or osteoblast lineage-specific Osteocalcin-Cre mice. Results from this study will elucidate the interactions between endogenous SNS outflow and bone remodeling during bone mass accrual and aging. This research will ultimately expand the understanding of age-related osteoporosis, and open new avenues of research towards treatment of bone metabolic diseases.

2017 FALL PHARMACOLOGY RETREAT

Wednesday, October 25, 2017

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3088 SMITH SPRINGS ROAD, NASHVILLE, TN 37013

KEYNOTE ADDRESS

Keynote Address

"A New Vision for Graduate Education at Vanderbilt"

Presented by Mark T. Wallace, Ph.D.
Dean, Graduate School

About Mark T. Wallace, Ph.D.

Mark Wallace earned his BA in biology in 1985 and his PhD in neurobiology in 1990 from Temple University. From 1990 to 1993, he conducted postdoctoral research at Virginia Commonwealth University School of Medicine, and subsequently served as assistant professor of physiology from 1993 to 1994. He joined Wake Forest University School of Medicine as assistant professor of neurobiology and anatomy in 1994 and was promoted to associate professor in 2002, a position he held until joining the Vanderbilt University School of Medicine.

Dr. Wallace joined Vanderbilt in 2006 as associate professor in the Department of Hearing and Speech Sciences and as associate professor of psychology. In 2008, he was promoted to professor of hearing and speech sciences, psychology and psychiatry and also named director of the Brain Institute. As director, Wallace oversaw neuroscience research activities and community outreach and development activities.

He was named associate director of the Silvio O. Conte Center for Neuroscience Research in 2010 and was awarded the Louise B. McGavock Endowed Chair in 2015. He is an investigator at the Vanderbilt Kennedy Center for Research on Human Development and a member of the Center for Integrative and Cognitive Neuroscience.

In January 2016, Dr. Wallace was named Dean of the Vanderbilt University Graduate School. In his role as dean, he serves as the chief administrator for all graduate programs within the university's schools and colleges. The Graduate School is the pathway and official school of record for graduate student applications, admissions, registration and enrollment; the monitoring and recording of academic progress and milestones; and the awarding of degrees. In addition, Dr. Wallace oversees graduate offices of diversity, professional development, placement and postdoctoral affairs.

In announcing Dean Wallace's appointment to his new role, Susan Wente, Provost and Vice Chancellor for Academic Affairs stated: "Mark has an aggressive vision for meaningful change at the Graduate School and a suite of innovative ideas on how to propel graduate education forward. He has a proven track record of creating environments where graduate students thrive, as well as dramatic success in building trans-institutional relationships and infrastructure. He also understands and values the importance of diversity to our future and is committed to expanding equity and inclusion at every level of the school. He is exactly the right person to make the vision and goals our students and faculty have articulated for graduate education a reality."