This essay reflects on my research activities since beginning my tenure-track appointment at MSU in the Neuroscience Program and Department of Physiology in January 2013 and outlines my plans to grow and expand my current independent research program. ***The overarching goal of my research program is to understand changes in the brain that underlie neuropsychiatric disorders.*** More specifically, my objective is to identify molecular mechanisms that mediate changes in ventral tegmental area dopamine neuron function that influence drug and mood behaviors. We utilize mouse models combined with biochemistry, molecular biology, and microscopy techniques to identify chronic drug- and stress-induced changes in gene expression and signaling in ventral tegmental area (VTA) dopamine (DA) neurons and then directly interrogate their effects on cell morphology and behavior via genetic and viral approaches. These experiments are significant, as the role of VTA DA neurons has now expanded far beyond their initial characterization in mediating the rewarding effects of abused drugs, and activity of VTA neurons has now been shown to play a critical role in myriad motivated behaviors including feeding, sex, and sociability. While the classification and circuitry of these VTA neurons has been at the forefront of neuroscience, the definition of the cellular mediators critical for changes in their structure and activity has lagged behind. Thus, the work of my lab fills an important niche, one that could be crucial to developing more effective therapies for reward-related disorders.

I have established a federally funded, vibrant, and growing research program at MSU. As evidence of this, I have obtained external funding (3 grants) to support two separate lines of work in the lab. The first was an R03 award from NIDA to develop a co-morbid mouse model of opiate use and mood disorders. This funding generated critical data that supported an R01 application (recently funded by NIDA) investigating the role of a specific signaling protein (TORC2) in this model. Additionally, this work is supported by a graduate student fellowship from the PhRMA Foundation. In a separate series of experiments, we are investigating the role of a novel candidate protein (SGK1) in drug addiction. These studies received initial support from a 1-year New Investigator grant from the PhRMA foundation and led to the first senior author publication from my lab, and the data generated was used to support a second R01 application that was reviewed this fall (October 3-4). I have also assembled a productive research group, with students in my lab responsible for two additional manuscripts submitted/in preparation. My lab is home to both graduate and undergraduate students who have been recognized for their excellence, earning travel awards from local and national organizations. My stature in the field is also growing, as I was recently chosen as a Discussion Leader for the Catecholamine Gordon Research Seminar and have been invited to submit a review on addiction brain circuitry. Together, these grants, awards, and publications highlight my progress towards establishing an internationally recognized research program.

Background and Significance

Although drug dependence and addiction are major health and economic burdens, our limited understanding of the underlying neurobiology limits better diagnostics and interventions. This is particularly problematic for opiate drugs, due to increases in both prescription and nonprescription use, and has resulted in a rapid increase in overdose deaths. The drive to consume drugs, and more generally to pursue any type of pleasurable experience, is dependent on signaling within the brain reward circuit, which originates in the VTA (Fig. 1). Dysregulation of the mesocorticolimbic reward circuit is known to contribute to various aspects of drug addiction, with alteration in the activity and output of DA neurons in the VTA contributing to the rewarding aspects of drug use. I have contributed significantly to this literature with my findings on structural changes induced in VTA neurons that contribute

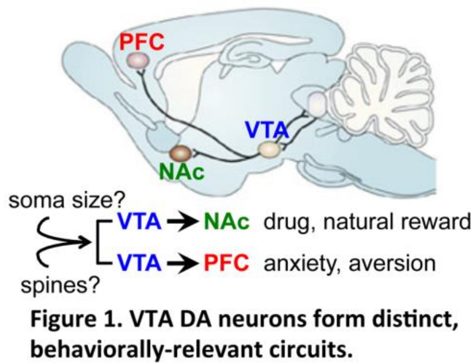

Figure 1. VTA DA neurons form distinct, behaviorally-relevant circuits.

to the phenotype [1, 2]. While our understanding of the molecular mechanisms in VTA DA neurons that mediate these cellular and behavioral effects is still limited, recent advances offer the promise to more selectively identify and assess the role of specific cells and circuits in drug-induced changes in animal behavior. It is increasingly clear that regulation of VTA activity is not only important for the acute, rewarding effects of drugs, but mediates myriad reward-related behaviors in both the presence and absence of drugs (Fig. 1). For example, knowledge of the contribution of VTA DA neuron dysregulation to mood-related disorders such as depression is relatively recent [3, 4], and may help to explain the significant comorbidity of substance abuse and mood disorders. However, while optogenetic studies have elegantly implicated VTA neuronal activity in many reward-related behavioral states, the molecular mechanisms that actually control this activity remain largely elusive. Thus, there is a critical gap in our knowledge of molecular mechanisms in VTA that are crucial for regulation of drug- and chronic stress-induced neuroadaptations. This has resulted in 3 main lines of research in my lab at MSU: 1) Determining drug-induced changes in VTA neurons; 2) Determining chronic-stress induced changes in VTA neurons; and 3) Determining the role of VTA in the comorbidity of drug and mood disorders. These projects will reveal novel nodes for pharmacological intervention to improve treatment of these devastating disorders.

My Work at MSU

1. Determine drug-induced changes in VTA neurons.

While regulation of VTA DA neuronal activity plays a clear role in drug reward, we have a limited understanding of the molecular players. My postdoctoral work sought to investigate mechanisms underlying a morphological change in VTA DA neurons correlated with opiate reward: a decrease in soma size. I identified this change in both mice and humans [1]. Moreover, I found that activity of a specific kinase complex, target of rapamycin complex 2 (TORC2), was decreased in the VTA following chronic opiate exposure, and that restoring function of this complex in VTA DA cells was sufficient to normalize VTA DA soma size and neuronal activity, and behavioral response to opiates. A limitation to these studies was that we could not classify the subtypes of VTA DA neurons, and it is now clear that there is cellular heterogeneity in the VTA and that VTA DA neurons form multiple distinct, behaviorally relevant circuits. Thus, my lab is now examining whether drug-induced changes in structural plasticity (soma size and dendritic spine density) occur in all VTA DA neurons, or are limited to functionally specific subsets of VTA DA neurons (Fig. 2).

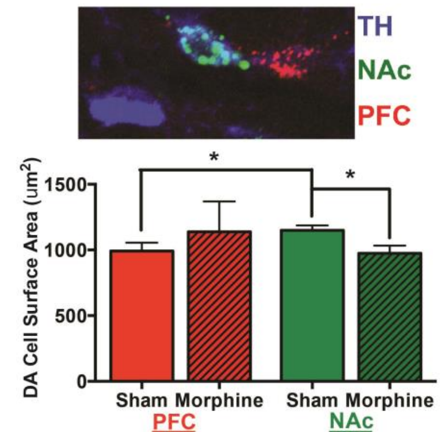

Fig. 2. Circuit-specific differences in VTA DA soma size. Lumafluor bead labeling of DA neurons (blue) that project to the nucleus accumbens (NAc, green) and pre-frontal cortex (PFC, red). Average DA cell soma size in sham or morphine treated-mice, NAc-projecting neurons (green) compared to PFC-projecting (red), n=3-5 mice, $^*p<0.05$.

Additionally, we are interested in identifying novel genes induced by drugs of abuse that underlie changes in VTA structure and activity. Using RNA-sequencing, we were able to identify differential gene expression patterns induced by cocaine and morphine in the VTA,

work that was recently published in the *Journal of Neurochemistry* [5]. We also identified a small number of genes that were similarly regulated by both drugs, including one that is highly upregulated, serum- and glucocorticoid-regulated kinase 1 (SGK1). Funding by the PhRMA foundation has supported this work, and we have now generated a series of viral constructs to modulate VTA SGK1 signaling via alteration of specific amino acid residues. We have found that catalytic inactivation SGK1 (or decreasing phosphorylation at a specific residue) specifically in the VTA decreases the voluntary intake of morphine in mice. These exciting data were the basis for a recently submitted R01 application (scored in Oct. 2016, 39%).

Finally, we are now improving upon these initial studies by utilizing innovative techniques such as Ribo-TRAP that allow cell-type specific analysis of gene expression. We have already generated intriguing data using this technique, as we show that the SGK1 increase induced by morphine does not occur in the VTA DA cells, but in the VTA GABA cells. We are now coupling Ribo-Trap with RNA-sequencing in order to define the cell-type specific transcriptome of VTA DA and GABA cells. This dataset will be a critical resource for the drug addiction field and will lead to multiple hypotheses for my lab to test in future studies. Critically, we will use genetic driver lines and conditional viral vectors to then test directly whether changes in specific signaling molecules are necessary and sufficient to alter drug reward and consumption.

2. Determine chronic stress-induced changes in VTA neurons.

In addition to studying the role of the VTA in cellular and behavioral adaptations induced by drugs, we have also established studies to examine the effect of chronic stress on the structure and function of VTA DA neurons. Chronic stress is a known risk factor in the development of mood and anxiety disorders, such as depression. We have recently found that a form of chronic stress termed chronic social defeat, which is used as a preclinical model of depression, induces similar morphological changes in VTA DA neurons to those induced by chronic opiate exposure. Thus, we are now conducting experiments to determine the molecular mechanisms in the VTA that are necessary for chronic stress-induced changes in VTA DA neuron structure and function.

3. Determine the role of the VTA in comorbidity of drug and mood disorders.

The last line of work in my lab addresses a known complication in the treatment of addiction; that there is a strong connection between drug use and mood disorders, with little known about the molecular mechanisms that may influence comorbidity, or susceptibility to both disorders. This knowledge gap is problematic, as rodent models of mood disorders often employ physical stressors that may confound study of pain-relieving opiate drugs. To overcome this hurdle, my lab has employed an innovative approach, using a purely psychological stress, chronic emotional stress, which allows the investigation of mouse opiate reward and consumption both during and following stress in the absence of physical trauma. The generation of this model was funded by an R03 grant from NIDA, and the expansion of this work to examine the role of TORC2 signaling in VTA DA neurons in stress-induced changes in opiate reward and consumption was recently funded by NIDA (R01). Emotional stress provides a unique developmental window to study the mechanisms that underlie behavioral changes, since there is only a modest phenotype one day post-stress that incubates to a more "depressive"-like phenotype one month later. This is a significant advance from current approaches, which examine reward changes after a peak stress response, and allows

for evaluation of how opiate intake impacts the development of depressive-like behaviors, potentially informing human comorbidity. Moreover, individual differences of mice to susceptibility to emotional stress allows us to address mechanisms of resilience, as well as sex as a biological variable, as female emotional stress has recently been reported. For example, we have shown a negative correlation between social interaction score and morphine intake: the more "depressed" the mouse, the greater the voluntary intake of morphine (Fig. 3). We are now using this model to address the hypothesis that VTA TORC2 signaling controls stress-induced changes in morphine reward and consumption and alters VTA DA neuron structure.

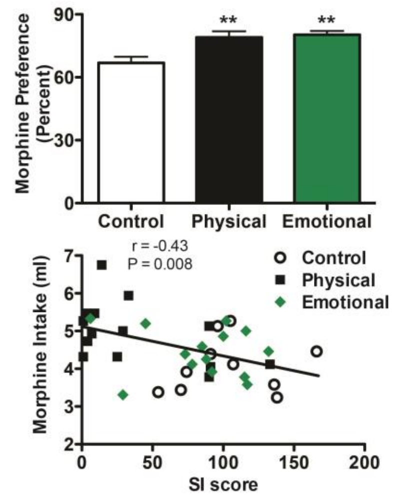

Fig. 3: Mice exposed to physical and emotional social defeat stress increase morphine preference and intake in a two-bottle choice assay. Morphine reward changes are long-lasting, as SI score is negatively correlated with morphine intake 14 days following stress.

Collaborations

I have been fortunate to establish collaborations with labs at MSU and at other institutions that have been beneficial to the growth of my research program. These collaborations have resulted in multiple co-author publications (and to additional funding opportunities. For example, is a co-I on my recently submitted SGK1 grant and is generating novel viral constructs utilized in both of my R01 grant applications. Additionally, I am part of a larger group led by that seeks to establish an MSU Center for Sex Differences Research through SPG funding. Collaborations in which I contribute my expertise in models of drug abuse and depression have also opened investigation of the role of the VTA in additional disease states, such as Parkinson's disease (as co-I on an R01 application recently submitted by and colleagues in TSMM) and Inflammatory Bowel Disease (through a multi-investigator Synergy grant submission to the Rainin Foundation with). Finally, much of the work in my lab is in some way a collaboration with my spouse, , which has been highly productive (10 co-authored publication). However, we are careful to give each other appropriate credit in our publications and the leader of each project is made clear in the author order, where listing as the first (during our post-docs) or final author (as independent investigators at MSU) denotes leadership.

Future Directions and Plans for Additional Funding

The studies described here are the foundation of an innovative research program to identify novel molecular mediators of drug addiction and depression in order to improve treatment. While the current funded grants and applications are focused on candidate molecules we have significant evidence are involved in either drug addiction (SGK1) or the comorbidity of opiate abuse and depression (TORC2), the cutting-edge cell-type specific genome-wide studies that my lab is now conducting will lead to novel hypotheses and multiple future avenues for growth. This, combined with collaborations that examine the role of the VTA in additional disease states, will expand my research program and firmly establish my niche as an expert in translationally relevant VTA neuroadaptations.

number of scheduled lectures to accommodate class periods dedicated to in-class activities that required students to work actively in groups to complete an assignment. I especially enjoyed this challenge, as I was able to generate an exercise that required the students to read and then answer a series of questions about cutting-edge papers in neuroscience that utilized many of the concepts I had covered during lectures. I felt that this activity, which provided the students a critical opportunity to interpret data from a primary research paper, also allowed them a glimpse of the exciting nature of neuroscience research, and how the basic concepts they were learning were critical to asking and answering the questions of today.

Given the success of such activities, and the students' positive response, we have restructured the NEU 301 course this year to more formally integrate active learning. Because the class has now grown beyond the ability to break up into groups during lecture periods (>250 students in 2016), the course now includes 2 lecture periods and 1 recitation session per week. These recitation sessions are limited to 50 students, allowing for increased interaction between students and instructors. Thus, this year I have had to reassess my learning objectives, cut down my lectures to the core concepts and develop weekly recitation activities that allow the students to apply these principles to gain a more complete understanding of the material. We have also introduced weekly online quizzes that have multiple benefits: 1) more frequent assessment of students' knowledge; 2) practice in test taking skills; 3) combined with recitation activities, additional opportunities for students to demonstrate mastery of the material in a variety of formats over time, instead of only during 2-3 exams. In addition to delivering lectures for the first half of this course, this year I am also serving as the Course Director. This is a valuable experience, as I am responsible for maintaining consistency across the course, managing the Teaching and Learning Assistants that assist with the recitations, and completing the considerable logistical and administrative duties that come with overseeing a large course. While the changing nature of the course has been challenging at times, I feel that it has also improved my teaching, as I have had to critically evaluate both the curricular content and the metrics we use to assess mastery with changing demands. Additionally, I have utilized feedback from student evaluations to further refine my technique and have received overall positive assessment of my portion of the course via SIRS.

Outside the classroom, I am actively involved in graduate and undergraduate mentorship. I currently have three graduate students (Dept. of Pharmacology and Toxicology, Neuroscience Program) in my laboratory, and have mentored a master's degree student (graduated 2014). I also serve on the thesis committees of 7 students from various programs. I currently have three undergraduate students in my lab, which not only allows me to teach students about conducting rigorous, ethical scientific studies, but allows for more personal connections and the opportunity to help each student identify their scientific passion and help them attain the skills they need to successfully pursue that goal. While I alter my specific approach to the needs of each student, I encourage all of my students to present their work as broadly and frequently as possible, and to apply for awards as recognition of their excellence. This mentoring occurs through formal weekly meetings, along with daily, less-structured interactions. I see mentoring as an extension of my teaching, where members of my lab have the benefit of a smaller close-knit setting that allows them a safe environment to grow and define their careers.