How does the brain regulate emotions? That is the question that drove me to pursue a career in neuroscience research. During my undergraduate education various experiences (see personal statement) shaped my career goal: to establish my laboratory in Puerto Rico in order to improve the research training opportunities for underrepresented students.

My first research experience was during my sophomore year at the University of Puerto Rico when I joined Dr. Gregory J. Quirk's laboratory. The Quirk Lab studies how the brain regulates emotions by using fear as a model system. Fear conditioning consists of pairing a tone with a shock such that subsequent presentation of the tone elicits a fear response, measured as freezing in rodents (immobility). Extinction of fear occurs by repetitive presentation of the tone without the shock. Fear makes a good model to study the underlying neural mechanisms of emotional regulation given the advantage of a stimulus that evokes a simple emotion, as opposed to other more complex emotions. My first project consisted in determining the roles of two medial prefrontal cortex (mPFC) subregions and the ventral hippocampus (vHPC) in fear memory and extinction. Initially, I was trained by a graduate student to perform the experiments but quickly developed enough independence to run experiments on my own. Using pharmacological methods to locally disrupt neural activity, we found that the prelimbic (PL) region of the mPFC is necessary for fear memory recall, whereas the neighboring infralimbic (IL) region is necessary for fear extinction. Pharmacological inactivation of vHPC disrupted both fear memory recall and extinction learning. We proposed a model based on anatomy in which vHPC modulates fear through PL projections and extinction through IL; this project turned into my first publication[1]. I presented my work during the annual Society for Neuroscience meeting. Interactions with other scientists at the meeting resulted in a new question: how are these different brain regions encoding representations of fear and extinction memories? This is why I became interested in electrophysiology and functional connectivity. Next semester, in preparation to understand functional connectivity I took a graduate course on electrophysiology and synaptic plasticity. I became fascinated with the idea of using electrophysiology to study how neural activity coordinates behaviors at all levels of complexity, from emotional regulation.

My interest in using electrophysiology to understand how the brain is encoding information led me to work with Dr. Christopher Moore at the Massachusetts Institute of Technology. There, I worked under the supervision of a graduate student on a project designed to determine how somatosensory perception is altered by artificially induced gamma oscillations in the somatosensory cortex of mice during behavior. Gamma oscillations emerge in states of attention and correlate with good performance during sensory tasks, but no one had tested if inducing gamma suffices for good performance. Using optogenetics to manipulate neural activity in behaving mice we were able to test this hypothesis. By expressing the light sensitive channel ChR2 in the somatosensory cortex, gamma oscillations can be generated by shining light in a temporally controlled manner. This experience shaped my current interest in manipulating circuits with optogenetics to determine if brain regions have causal relationships regulating anxiety, and it also influenced my undergraduate thesis work in the Quirk Lab. Learning that the thalamus is a relay station to the sensory cortex, sparked my curiosity about thalamic interactions with mPFC. Although much is known about thalamocortical interaction in

the sensory cortices little is known about how the thalamus interacts with higher order cortices such as mPFC. Upon my return to University of Puerto Rico I started my undergraduate thesis on the role of the thalamus in fear and extinction. The dorsal midline thalamus (dMt) projects strongly to mPFC and to other brain regions involved in fear: vHPC and the amygdala. I hypothesized that dMt could be regulating fear through inputs to any of these regions. Pharmacologically inactivating dMt prior to fear conditioning or immediately after the learning had no effect, but inactivation 24 h after the fear learning impaired recall of the memory. This suggests that dMt becomes part of the fear memory circuitry slowly after learning occurs. Moreover, we found that dMt inactivation affected neuronal activity in the amygdala during fear memory recall, suggesting that dMt modulates fear recall by affecting downstream amygdala activity. This work became my first first-authorship publication[2].

Once I started graduate school I knew I wanted to focus my efforts in learning electrophysiology. In order to learn single cell in-vivo electrophysiology, I rotated in the laboratory of Dr. Randy Bruno. The Bruno lab studies thalamocortical dynamics. Specifically, I was studying the role of spike synchrony in sensory stimuli encoding across the cortical layers. It's been shown that activity of thalamic pairs becomes correlated when stimuli is present, suggesting that timing of spikes is important for encoding of stimuli. Given this previous work we predicted that cortical pairs that receive thalamic input might also become correlated with stimuli presentation in order to propagate the signal from thalamus to cortex. To test this I was recording pairs of neurons in different layers of the somatosensory through a craniotomy window. The purpose was to assess if stimulus presentation increased the correlation of the activity of neuronal pairs and to see if the cortical laminar location of neuronal pairs influenced their correlation level. This project is still ongoing in the Bruno Lab. This rotation project was essential for my confidence performing in vivo extracellular recordings and analyzing electrophysiological data.

As a graduate student in Joshua Gordon's laboratory, I am using extracellular recordings and optogenetics to study activity of both single neurons and populations of neurons across different brain regions during low and high anxiety states. I was drawn to study anxiety because it has close overlap with circuitry I have been studying in the Quirk Lab. Like fear learning, anxiety is dependent on mPFC and hippocampus. It is fascinating how one circuit has the flexibility to mediate multiple behaviors. Using the elevated plus-maze a well-characterized anxiety assay, I will determine the role of the hippocampal input to mPFC generating anxiety-like behavior in mice (see proposal).

[1] Sierra-Mercado D., **Padilla-Coreano N.** and Quirk G.J. *Dissociable roles of prelimbic, infralimbic, ventral hippocampal, and basolateral amygdala areas in fear expression and extinction memory .Neuropsychopharmacology, 2010*.

[2] **Padilla-Coreano N.,** Do Monte F.H. and Quirk G.J. *A time-dependent role of midline thalamic nuclei in the retrieval of fear memory.* Neuropharmacology, August 2011.

It was in front of my piano where I was first mesmerized by the brain. Ever since I was child, I have experienced music's emotional effects when Chopin's nocturnes made me cry and Mozart's sonatas cheered me up. During high school, while reading excerpts of a biology textbook I discovered that the magic comes from the brain and not the heart. My scientific curiosity awoke, and new questions emerged. How do we perceive sounds? How does the brain decide what is just noise and what are melodic sounds? And how do sounds evoke such strong emotions? I started college with a quest to understand how the brain works. During this quest I developed a desire to become leader of the neuroscience research community and to mentor students in my own laboratory in Puerto Rico.

During my sophomore year I joined Dr. Gregory Quirk's laboratory in the department of Psychiatry at the University of Puerto Rico (UPR). After one semester learning to do behavioral experiments and pharmacological manipulations in rats, I quickly discovered the beauty of using a simple behavioral model-Fear Conditioning- to study how the brain regulates emotion. The Quirk lab focused on understanding how two brain regions: prefrontal cortex (PFC) and amygdala interact during fear conditioning. My interest in how PFC regulates emotions increased with my increasing knowledge of neuroscience and lab experience. During the time I worked on my undergraduate thesis, I frequently found myself standing at the white board in my mentor's office drawing diagrams and debating potential experimental outcomes. By the end of my junior year I published my first research article and already knew that I was headed to graduate school to continue to study how the brain regulates emotions.

In addition to the experimental side of research, I learned that as a scientist I have a role in society that goes beyond the bench work. As my research experiences expanded- conferences, internships, seminars- I realized that my undergraduate institution lacked (tense?) a rigorous research community. Students graduated without a good research experience and almost no one applied to graduate school. I discovered I was one of the few lucky that received research training in a qualified lab thanks to the commitment of Dr. Gregory Quirk[1]. The mentoring and challenge I felt as an undergraduate in the Quirk lab shaped my passion for research and my desire to mentor students, especially students with limited access to training opportunities. Every year during Brain Awareness Week, together with other lab members, I visited multiple public schools in the countryside of the island. This is a week when neuroscientists around the world leave lab and engage with the community to raise awareness about neuroscience research. The purpose of our school visits was to teach about the brain in interactive ways with the students and to discuss opportunities available for students in the sciences. During those school visits I felt happy and fulfilled by giving advice to students interested in pursuing a science career since I never received such advice, or met a scientist prior to entering college. Upon graduation, I decided to pursue my doctoral degree at Columbia University where, besides receiving excellent scientific training, I organize neuroscience workshops for K-12 classrooms. I form part of a group of scientists known as Columbia University Outreach Program (CUNO)[2] committed with bringing research into classrooms. Our goal is to bring scientists to classrooms to improve science in schools. We use neuroscience lessons prepared by graduate students with help of experienced teachers. Thanks to our commitment and excitement, we reached out close to 1000 students during the past academic year. My contribution to CUNO arises from realizing that science is more intuitive for layman in their native language. A year ago, I presented my research at the University of Habana, Cuba. Giving the presentation in Spanish, my native language, made the experience transcendental. After practicing the research talk with family and friends, it was evident that they engaged because there was no language barrier. This experience motivated me to make neuroscience more approachable for those who are not fluent in English. Through CUNO, I have directed my workshops to dual-language schools. New York City has a lot of

1 http://www.md.rcm.upr.edu/quirk/Home.html
2 http://blogs.cuit.columbia.edu/cuno/

Hispanic immigrants children and some public schools have classrooms for students who are not yet fluent in English. Conversations with the teachers gave me confidence that this visits impacted the students positively. It is inspiring to serve as a role model from early stages of my scientific career. I am confident that by educating children and getting them excited about science we are helping build the next generation of scientists, with simple classroom visits graduate students are making this happen in NYC.

Teaching is another way I will help build the next generation of scientists. My intellectual curiosity about music and the brain never ceased. That is why I decided to combine my passion for neuroscience and art to educate undergraduates. Columbia University allows graduate students interested in teaching to design undergraduate courses. I developed a seminar where students will learn about neuroscience from an artistic perspective. We will explore how art is perceived in the brain, how art evokes pleasure and emotions, and the interaction of psychiatric diseases and art. During the course students will learn about methods and techniques used in neuroscience research to critically discuss current research in cognitive neuroscience and art. Moreover, we will discuss how human research and animal model research shows overlaps in findings regarding emotional regulation and pleasure in the brain. During this part of the class I will bring students to my lab and other neuroscience labs to learn about different experimental setups. My course will be review by a committee of experts and I will teach it the next academic year. In preparation for teaching this class, I am taking a graduate seminar on the science of teaching. The experience of designing the course improved my communication skills and forced me to visualize the big picture of my own research.

My early passion for music led me to be curious of how PFC circuitry regulates emotions and higher order functions, and to seek out research training opportunities in neuroscience. Specifically, I decided to pursue my PhD in neuroscience at Columbia University in Dr. Joshua Gordon's laboratory. Currently, I am applying optogenetics and electrophysiological techniques to understand how the hippocampus, amygdala and PFC interact during anxiety behavior (see proposal). Moreover, I am gaining teaching experience while educating about neuroscience and research. At the end of my graduate, and eventually post-doctoral, training I will return to Puerto Rico to establish my research laboratory. In Puerto Rico, I will use optogenetics and electrophysiology to study emotional regulation while training students with the hope that they continue a research career, just like I have done.

Intellectual merit: **My research will help understand how brain regions interact during anxiety states. Understanding the mechanisms of normal anxiety states will help improve the treatments for anxiety pathophysiological states.**

Broader impacts: **My efforts will increase scientific training opportunities for underrepresented communities and improve the existing neuroscience community in Puerto Rico.**

1 http://www.md.rcm.upr.edu/quirk/Home.html
2 http://blogs.cuit.columbia.edu/cuno/

Charles Darwin was the first to hypothesize that emotions may serve as an important adaptive biological mechanism to avoid pain and seek pleasure. One of these emotions, anxiety, helps organisms avoid environmental threats. Neuroscientists have used lesion and pharmacological studies to identify brain structures involved in anxiety, but these manipulations lack temporal and spatial specificity. The recent development of optogenetic tools allows precise manipulation of neurons in these brain structures in order to test their causal roles in generating anxiety behavior. Recent work from our group has shown that neural activity from the ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC) correlates with anxiety-like behavior in mice. Such correlative studies do not address the causal role of the connection between these structures**. I will test the hypothesis that vHPC input into the mPFC is required for anxiety and for mPFC neural representations** in two specific aims**:**

Aim 1: Using optogenetic silencing and anxiety assays, I will test if neural activity from the vHPC-mPFC circuit is required for anxiety behavior.

Aim 2: Combining optogenetic silencing and electrophysiological recordings, I will test if the vHPC input to mPFC is required for forming a neurophysiological representation of anxiety in the mPFC.

Background: The first reports implicating the mPFC and the vHPC in anxiety come from studies showing that lesions of these brain structures reduce anxiety in rodents[1,2]. Interestingly, the vHPC makes robust unidirectional projections to the mPFC, suggesting that vHPC input to mPFC may be part of a distributed brain circuit that contributes to anxiety behavior. In support of this, work from the Gordon lab shows that local field potentials (LFPs, a measure of population synaptic activity) in the mPFC and vHPC become highly correlated in the theta frequency (8-12 Hz) when mice are tested in anxiety assays[3]. Moreover, changes in anxiety behavior correlate with mPFC activity, specifically the power of the theta frequency in the LFPs. This correlative evidence, however, does not prove that vHPC-mPFC circuit has a causal role in generating anxiety behavior**.** Further work from the Gordon lab has correlated single-neuron activity in the mPFC with anxiety behavior. A large fraction of mPFC neurons show consistent firing rates in within anxiogenic spaces and within safe spaces[4] .Moreover, in mPFC neurons, firing rates predict anxiety behavior such that changes in firing rates occur before mice transition from safe to anxiogenic environments and vice-versa. Finally, these mPFC neurons are synchronized to vHPC LFPs in the theta frequency, suggesting that vHPC input plays a role in generating this activity. I will directly test if vHPC input has a causal role generating the firing patterns related to anxiety in mPFC neurons. **Approach**: Optogenetic tools allow precise manipulation of neuronal populations by expressing genes of light-activated proteins that can either excite or inhibit neurons. One of these genes is Archaerhodopsin-3 (Arch). Arch codes for a protein that is sensitive to green light. When Arch is expressed in neurons, upon activation it hyperpolarizes neurons, which causes inhibition of neural activity. I will use Arch to silence the vHPC-mPFC circuit and assess the effects on anxiety behavior.

Assaying anxiety in mice: While it is difficult to measure anxiety in mice, it is possible to measure avoidance behavior in the presence of potential danger. I will use two well-characterized anxiety tests: the elevated plus maze (EPM) and the open field. The EPM has open and closed arms, and mice generally avoid the open arms, due to vulnerability to potential predators in open and brightly lit areas. Thus, avoidance of the open arms is a measure of anxiety-like behavior. Similarly, in the open field assay, mice generally stay in the periphery and avoid the center. Importantly, drugs that are decrease and increase anxiety in humans decrease and increase, respectively, avoidance in these anxiety assays.

References: [1] Kjelstrup, Tuvnes, Steffenach, et al. *PNAS.* (2002) 16, 10825-30 **[2]** Deacon, Penny, Rawlins. *Behavioural brain research.* (2003) 139, 139-55 **[3]** Adhikari, Topiwala, Gordon. *Neuron*. (2010) 65, 257-69 **[4]** Adhikari, Topiwala, Gordon. *Neuron*. (2011) 71, 898-910 **[5]** Tye K., Prakash R., Kim S. et al. Nature (2011) 471, 358-62

Aim 1: To assess the behavioral effects of silencing vHPC -mPFC circuit during anxiety assays. I hypothesize that bilaterally silencing vHPC projections to the mPFC will decrease anxiety-like behavior. I will test this hypothesis by expressing Arch in the vHPC through a viral vector. I will silence the vHPC projections to the mPFC by delivering green light in axonal terminals in the mPFC. The use of Arch to silence axonal terminals has worked in recent studies[5] and is being used successfully in the Gordon lab. In a control group, I will inject vHPC with a viral vector containing only the inert flourophore, mCherry. In both cohorts of mice I will implant an optical fiber for localized light delivery while mice behave in the EPM and open field test. The use of two different anxiety assays provides a robust way to confirm that the vHPC-mPFC is indeed involved in anxiety behavior. During bilateral photostimulation of vHPC terminals in the mPFC, I expect to see decreased avoidance of open spaces in the EPM and the open field in the cohort injected with Arch, but not in the control cohort. On the other hand, I expect that unilateral photostimulation will not be sufficient to decrease anxiety, given that the vHPC projections to mPFC are contained within the same hemisphere. **Interpretation and alternatives**: If avoidance decreases during photostimulation, then this would confirm that vHPC-mPFC circuit is necessary for anxiety behavior. On the other hand, if avoidance is not affected then, it suggests that the vHPC-mPFC circuit does not have a causal relationship in generating anxiety behavior. One caveat is that it is possible that the vHPC inputs are not completely silenced by Arch activation. Alternatively, the amygdala is another region shown to be involved in anxiety behavior. The amygdala projects to both mPFC and vHPC, so it is possible that projections from amygdala to both the vHPC and the mPFC or other regions have a causal role in generating anxiety behavior.

Aim 2: To assess the electrophysiological effects of silencing the vHPC-mPFC circuit in mPFC single cell activity during anxiety behavior I hypothesize that silencing the vHPC-mPFC circuit will disrupt the consistency of mPFC firing rates in safe vs. anxiogenic environments. To silence the vHPC-mPFC circuit I will use the same approach described in Aim 1. In addition to implanting an optical fiber in mPFC, I will implant multi-channel electrodes to record single-unit neurons in mPFC while mice are tested in the EPM and open field. I will unilaterally silence vHPC-mPFC circuit by delivering the light to only one hemisphere. This way it is likely that anxiety behavior will be unaffected and yet changes induced by silencing vHPC input can be assessed in mPFC neurons. I will record mPFC neurons while testing anxiety behavior in the EPM and the open field test. I expect that silencing the vHPC projection to mPFC will disrupt the specific firing correlation seen in the safe vs. anxiogenic environments in the EPM and open field test. **Interpretation and alternatives:** If silencing the vHPC-mPFC circuit disrupts the firing patterns of safe vs. anxiogenic environments, then the input of vHPC to mPFC has a causal role in providing necessary information for building an anxiety representation in mPFC single units. Alternatively, if the mPFC firing patterns are unaffected, then it is possible that the input to the mPFC from other regions, such as the amygdala, has a role building this mPFC anxiety representation. This case would suggest that anxiety behavior and anxiety representation in mPFC are controlled by different circuits: the vHPC-mPFC circuit controls the anxiety-like behavior, while the amygdala-mPFC circuit controls the representation of anxiety in the mPFC. **Broader impacts:** This work will improve the standing knowledge of the role of vHPC and mPFC activity in anxiety behavior. Understanding the circuitry of anxiety brings us one step closer to understanding the mechanisms of emotional regulation. Moreover, this know will help the development of novel, more effective treatments for anxiety-related disorders.

References: [1] Kjelstrup, Tuvnes, Steffenach, et al. *PNAS*. (2002) 16, 10825-30 **[2]** Deacon, Penny, Rawlins. *Behavioural brain research.* (2003) 139, 139-55 **[3]** Adhikari, Topiwala, Gordon. *Neuron*. (2010) 65, 257-69 **[4]** Adhikari, Topiwala, Gordon. *Neuron*. (2011) 71, 898-910 **[5]** Tye K., Prakash R., Kim S. et al. Nature (2011) 471, 358-62