Steve Ramirez, PhD

Aim of Research Program

My research program is guided by two major questions:

1. ***How do neural circuits process discrete positive and negative memories?***
2. ***How does acute and chronic manipulation of memories modulate stress-induced maladaptive states at the cellular, circuit-level, and behavioral levels?***

The overarching mission of my research is to understand the basic mechanisms governing memory formation and thereafter to artificially modulate memories to reverse and prevent psychiatric disease-like states in rodents. In mice and humans, the hippocampus (HPC) has been implicated in processing positive and negative memories as well as in modulating stress-related states. Moreover, chronic stress affects numerous brain areas involved in memory, emotion, and motivation, including the HPC, prefrontal cortex (PFC), and amygdala (BLA); it abnormally alters a variety of cellular events, including neuronal morphology, gene expression patterns, and neurogenesis; and, it can precipitate several maladaptive states, such as depression- and anxiety-like behaviors. Traditionally, reversing these conditions has relied on drug-based interventions, which by their nature produce brain-wide non-specific effects and rely on drugs that are iterations of—and without improved efficacy over—their 1960s counterparts. To that end, at Boston University's Department of Psychological & Brain Sciences, **by utilizing an innovative and intersectional approach my research first seeks to identify and manipulate the brain's endogenous plasticity mechanisms by means of memory activation to resolve underlying principles organizing memory formation, and secondly to demonstrate its therapeutic potential for lastingly alleviating cellular, circuit-level and behavioral abnormalities comprising psychiatric disease-like states.**

Qualifications for The Proposed Research Program

My training has prepared me to lead a diverse research group to understand the neural circuits that underlie memory and complex behaviors. I have an exceptionally strong background in animal behavior (in both mice and rats), immunohistochemical techniques, and circuit probing strategies including optogenetics, chemogenetics, and viral gene transfer. My graduate work, conducted in the laboratory of Dr. Susumu Tonegawa at MIT, focused on identifying and manipulating cells in real-time that processed discrete memories. To achieve this, we developed a genetic tagging system for visualizing and optically manipulating brain cells previously active during memory formation. Our work first demonstrated that cells in the HPC that were previously active during fear learning were sufficient to drive the behavioral expression of the associated memory. These HPC cells were also shown to undergo plasticity-related changes specifically during learning and are necessary for memory recall, thus supporting their mnemonic nature. Next, I leveraged these findings and demonstrated that delivering aversive or rewarding stimuli while simultaneously activating HPC cells that previously processed neutral memories was sufficient to artificially associate the neutral memory with each stimulus. Further, I demonstrated that optogenetically activating cells that previously processed positive memories was sufficient to alleviate stress-induced impaired motivation and anhedonia and was contingent on intact activity between the BLA and PFC. Finally, my work established a novel chronic stimulation protocol to confer antidepressant-like effects at the neuronal and behavioral levels by repeatedly stimulating positive memories in animal models of psychiatric disease-like states.

Due to my great fortune as an undergraduate and especially as a graduate student, I had an unusually rapid education with regards to learning and performing first-rate neuroscience. In 2015, I became a Principal Investigator and Junior Fellow at Harvard, where I have extended my expertise to include studies of *in vivo* and *in vitro* brain-wide circuit function in response to acute and chronic manipulation of positive and negative memories. My lab's ongoing work focuses on optogenetically and chemogenetically activating and silencing cells in the dorsal and ventral HPC to test whether or not active ensembles in each subdivision process independent features of memories. In a recently submitted manuscript, we show that chronic activation of dorsal HPC cells that previously processed a negative memory is sufficient to induce context-specific memory extinction, while the same manipulation in the ventral HPC is sufficient to induce context-specific memory enhancement. By discovering two nodes sufficient to bi-directionally modulate memory, we hypothesize that our light-induced extinction effects rely on the integrity of defined sets of "extinction neurons" in the BLA and PFC, while our light-induced enhancement effects rely on the integrity of defined sets of "fear neurons" in the BLA and PFC. Our ongoing experiments aim to test whether or not such manipulations can prevent the return of fear, e.g. spontaneous recovery, in animal models of post-traumatic stress disorder (PTSD), as well as to characterize the underlying neuronal landscape *in vivo* supporting each phenotype.

Ongoing and Future Aspects of my Research Program

By combining histochemical, *in vivo* imaging, optogenetic, and behavioral techniques, my work will interrogate the circuits that encode discrete memories and thereafter determine the precise cellular mechanisms by which these processes are dysregulated in maladaptive states. My work is funded by an Early Independence Award (DP5) from NIH, a NARSAD award, and a Family Foundation award.

PROJECT 1: Identify the contributions of dorsal and ventral HPC in driving emotional memories.

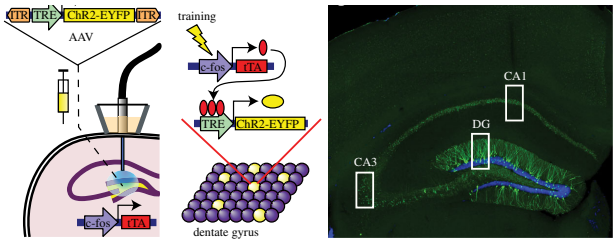

Figure 1. Genetically engineering hippocampus cells active during learning to express ChR2.
(A) A mouse is injected with a virus cocktail consisting of c-fos-tTA and TRE-ChR2 into the hippocampus, followed by optic fiber implants. When off Dox, the formation of a memory induces the expression of tTA, which binds to TRE and drives the expression of ChR2, thereby labeling a population of activated cells (yellow).

In both rodents and humans, emotional memories drive a variety of motivational and cognitive states, and yet the underlying circuitry sufficient to activate such memories remains poorly understood. Moreover, these states are directly impaired by chronic stress, which is a potent risk factor thought to produce symptoms of blunted or exaggerated emotions associated with major depression and anxiety disorders. In the service of answering Major Question 1, my recent work suggested that activating positive memories via the dorsal hippocampus was sufficient to promote motivation and hedonic-like activity. Our manipulations, however, did not affect approach-avoidance behaviors, thus motivating our search for key nodes in the HPC sufficient to differentially activate behavioral responses to positive and negative memory stimulation.

To that end, a myriad of anatomical and behavioral studies suggest that the HPC can be structurally and functionally segregated along its dorsal-ventral axis. For instance, dorsal and ventral HPC have distinct input and output connections and lesions of the ventral HPC have revealed its prominent role in regulating emotional, social, and avoidance behaviors. As such, ***I will first test the hypothesis that dorsal and ventral HPC both encode information about positive and negative stimuli that can then be used to bi-directionally modulate behavioral states***. Male and female mice will be injected with my recently developed virus cocktail which combines the activity-dependent nature of the c-Fos promoter with the light-sensitive properties of channelrhodopsin-2 (ChR2). The cocktail consists of AAV_9-c-Fos-tTA and AAV_9-TRE-ChR2-mCherry, which enables the tagging of cells active specifically during positive or negative memory formation (Figure 1). To test whether or not mice find stimulation of positive or negative memories intrinsically rewarding or aversive, they will first be administered a place preference and place aversion test. Upon entering only one side of a two-sided chamber, all mice will receive optogenetic stimulation of positive or negative memory cells; we hypothesize that the former will induce a place preference while the latter will produce place aversion to the stimulated side of the chamber. In the next experiment, and after tagging cells active during a positive experience, all mice will undergo a chronic stress protocol (e.g. social defeat) to produce depression- and anxiety-like phenotypes. To test if activated positive memories can reverse the effects of stress on hedonic activity and sociability—two core impairments in depression and anxiety—mice will undergo a battery of behavioral assays, including sucrose preference and social interaction tests. To test if activated negative memories can elicit anhedonia and social impairments, a negative memory group will receive optical stimulation during the same behavioral assays. Promisingly, my lab's preliminary data suggest that activated ventral HPC positive or negative memory cells are sufficient to decrease or increase avoidance behavior, respectively (Figure 2). Further, by using Designer Receptors Exclusively Activated by Designer Drugs (DREADDS), we will be able to elicit or inhibit downstream signals, such as BLA and PFC activity,

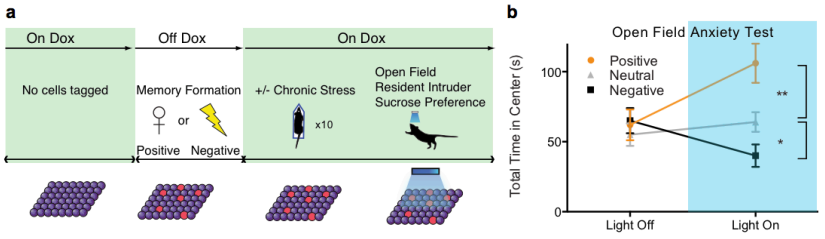

Figure 2. Reactivating ventral HPC-mediated memories bi-directionaly control anxiety-like behavior
(A) Behavioral schedule. Animals are kept on dox to prevent tagging of active cells with ChR2. Next, they are taken off dox and cells active during the formation of a positive (female symbol) or negative (lightning bolt) memory are labeled by ChR2. The positive memory group then undergoes a chronic stress protocol, followed by a battery of behaviors, during which ChR2-positive cells are activated. The negative memory group undergoes the same protocol. **(B)** Ventral HPC cells active during the formation of a positive (orange), but not neutral (grey), memory are sufficient to induce anxyiolysis in the open field test when optically reactivated, while negative (black) memory bearing cells are sufficient to induce anxiogenic-like behavior.

while simultaneously activating positive or negative memories to determine causal relationships between these neural signals and associated behaviors.

Second, I will perform brain-wide analyses to identify the cellular and physiological loci mediating positive and negative memories reactivation. By combining immunohistochemical strategies and cutting-edge *in vivo* calcium imaging techniques, we will test the hypothesis that positive memory stimulation elicits robust activity, as measured by c-Fos expression and increased calcium transients, in various areas associated with reward, including the amygdala and nucleus accumbens; conversely, we predict that negative memory stimulation will elicit robust activity in areas associated with aversion, including the central amygdala and habenula. Collectively, these experiments will address Major Question 1 by providing a novel framework for activating and visualizing memories in a brain-wide manner to bi-directionally modulate behavioral outputs.

PROJECT 2: What is the causal role of HPC outputs in driving independent features of behaviors?

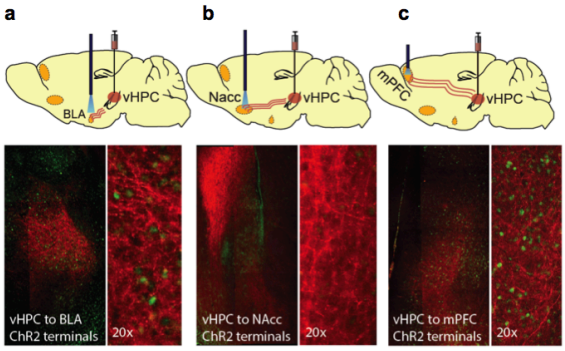

Figure 3. Labeling ventral hippocampus axonal terminals processing discrete memories
(A-C) A virus cocktail consisting of c-fos-tTA and TRE-ChR2-mCherry was injected into the ventral hippocampus to label cells active during memory formation and optic fibers were subsequently placed bilaterally over the BLA (a), NAc (b), or PFC (c). To the right of each panel is a 20x magnified view of the terminals in each area, showing robust ChR2-mCherry expression.

Here, I aim to gain causal insight into the information processed in target-specific HPC axonal terminals. Then, I seek to leverage this fundamental understanding of HPC output processing to study how stress impacts positive or negative memory-bearing HPC outputs and thereafter to reverse stress-induced changes at defined axonal terminals. **Overall, these experiments seek to disassemble positive and negative memories into their component terminals—which route distinct mnemonic information to various target areas—to differentially modulate separable features of behaviors.** Mice will be injected in ventral HPC with the virus cocktail from **Project 1**, followed by positive or negative memory-induced labeling of HPC terminals in BLA, PFC, and the nucleus accumbens (NAc)—these three regions have emerged as candidates in modulating social, avoidance, and reward-related behaviors, though how these HPC outputs carrying mnemonic information causally contribute to assembling a behavioral state remains untested. In the service of Major Question 1, and to reveal the role of each circuit element, each target-specific terminal first will be physiologically assessed and independently modulated to test for their capacity to drive the aforementioned behaviors. Additionally, in the positive memory group, I predict that a chronic stress protocol will depress, or decrease the activity of, terminals in each area, while in the negative memory group terminals will be enhanced, or potentiated, in each area. ***To further test the hypothesis that HPC outputs differentially process positive and negative mnemonic information,*** I will use optogenetics to correct the activity of HPC outputs post-stress. Recent studies have provided direct links between monosynaptic HPC outputs in modulating a battery of fear and reward-related behaviors; however, and importantly, these HPC studies utilize general promoters to drive ChR2 and indiscriminately label the majority of cells without differentiating between their mnemonic content and leave the behaviorally relevant information transmitted to areas such as the BLA, PFC, and NAc unknown. Thus, we seek to measure the causal contributions of HPC terminals *that directly process memories* in regions known to influence avoidance, reward, and social behavior. To correct the effects of stress at a specific HPC projection, animals in which stress induces aberrant increases, or potentiation of, activity at these terminals will undergo an optogenetic long-term depression protocol; conversely, animals in which stress induces decreases, or depression of, activity at each defined terminal will undergo an optogenetic long-term potentiation protocol. All groups will be tested in the aforementioned behaviors to measure if optically resetting activity at each terminal alleviates unique features of behavior. Promisingly, my preliminary data suggest that our novel virus cocktail expresses robustly both in HPC cell bodies and terminals in all three downstream structures (Figure 3), thus enabling structural and functional analyses of their impact on downstream activity and behavior.

Additionally, recent advances in viral technology have greatly enhanced our ability to trace circuits *in vivo*. ***I will then use circuit tracing techniques and confocal imaging to determine if HPC cells processing discrete memories send different outputs to target areas***. Separate groups will be prepared in which retrograde viruses (e.g. pseudorabies viruses), which jump one synapse back from the cells that they

infect, will be used in BLA, PFC, and NAc. In conjunction, given the anterograde labeling properties of TRE-ChR2-mCherry, we will then tag HPC monosynpatic projections processing positive or negative memories. We hypothesize that positive memory bearing HPC terminals preferentially synapse onto a subset of NAc cells that themselves preferentially project to areas involved in reward, such as the ventral tegmental area, while separate negative memory bearing HPC terminals may target PFC cells that preferentially project to areas involved in anxiety, such as the BLA. These experiments will enable a systematic evaluation of potential topography extant in HPC mnemonic-carrying terminals in a target-specific manner. Thus, **Project 2** aims to modulate independent features of memories and will help resolve the computations performed at defined terminals as well as their causal contributions in regulating circuit-level processes and behavioral outputs.

PROJECT 3: Determine if chronic activation of positive or negative memories results in stress resilience or susceptibility and identify the underlying physiological signatures.

While crude strategies exist for reversing cognitive and behavioral maladies, preventative strategies remain scarcer and with fewer delineated biological mechanisms. To address this challenge, and in the service of Major Question 2, I will test if chronic reactivation of positive memories prior to stress is sufficient to induce resilience by lastingly reprogramming neuronal circuits and behaviors, followed by measuring the underlying brain-wide neuronal and physiological mechanisms supporting memory's putative prophylactic capacity. A parallel set of experiments with similar analyses will test if chronic activation of negative memories is sufficient to mimic stress susceptibility. Our extensive preliminary data, contained in my recently submitted manuscript, show that chronic activation of negative memories offers a novel method of promoting fear extinction and enhancement of context-specific memories, and thus provides experimental validation that our chronic stimulation protocols are sufficient to enduringly modulate a variety of behaviors. ***Here, I will first stimulate positive and negative memories chronically prior to stress and assay for resilience and susceptible phenotypes.*** For instance, a positive memory-mediated resilience phenotype would manifest as a *lack* of stress-induced impairments in sociability, hedonic-like activity, and approach-avoidance behaviors, where as negative memory-mediated susceptibility would *mimic* the effects of stress in these behaviors. We will then identify histological candidates supporting resilience phenotypes and hypothesize that chronically activating positive memories will prevent the stress-induced atrophy (i.e. prevent dendritic spine decreases) and hyperactivity (i.e. prevent increased baseline c-Fos expression and calcium transients) observed in areas such as HPC, PFC, and BLA. Finally, lead by the histological analyses, ***I will inject our recently developed AAV_9-TRE-RCaMP6f virus along with AAV_9-c-Fos-tTA in candidate areas to label cells in an activity-dependent manner, which can then be subsequently monitored throughout our behavioral schedule***. This novel cocktail permits real-time imaging of calcium transients specifically in cells that were previously active during memory formation across a variety of brain areas, such as the BLA and PFC, and throughout our behavioral schedules. We will record activity *in vivo* before and after chronic positive memory stimulation both at baseline and during the aforementioned behaviors to note changes in response kinetics and activity dynamics, thereby providing physiological markers and targets for preventative strategies with unprecedented spatial-temporal resolution.

Potential for Collaborative Projects and Departmental Fit

These lines of research incorporate my background in cellular and behavioral neuroscience with my broadening technical skillset with particular focus on *in vivo* optogenetics, immunohistochemistry, and viral strategies. I also have growing expertise in molecular biology and *in vivo* imaging approaches. The proposed studies have great potential for funding via a number of external governmental and private foundation sources, as they are integrative, multidisciplinary, and innovative. As can be seen by my publication record, I have developed a broad network of researchers spanning multiple institutions, which has been instrumental to my success thus far and will allow for my continued success as I transition into an independent faculty position. My expertise provides a unique opportunity to collaborate with a wide range of faculty within Boston University's Department of Psychological & Brain Sciences.