(A) Face Sheet

BRAIN & BEHAVIOR RESEARCH FOUNDATION
Awarding NARSAD Grants

BRAIN & BEHAVIOR RESEARCH FOUNDATION
YOUNG INVESTIGATOR GRANT FACE SHEET

Applicant Information
(Please fill out all relevant information below)

Principal Investigator	Steve Ramirez, Ph.D.
Position Title	Principle Investigator, Junior Fellow
Institution	Harvard University
For MD Applicants, year residency was completed:	**For PhD Applicants, year degree was awarded:** 2015

For MD/PhD applicants please provide both years in the above designated areas.

Mentor/Institution (***Please list primary mentor name first***)	Joshua R. Sanes, Ph.D.
Number of peer-reviewed papers (Total/first authored/last authored):	8/5/0
Please identify all BBRF Scientific Council Members who will be in conflict with your application (i.e. your mentor, collaborators, same institution etc.) Complete list of members can be found here. ***If there is no conflict please list n/a**	Bernardo L. Sabatini, M.D., Ph.D. Takao K. Hensch, Ph.D.

Specialized Population

- ☐ Addiction
- ☐ ADHD
- ☑ Anxiety Disorders
- ☐ Bipolar Disorder
- ☑ Depression
- ☐ OCD
- ☑ PTSD
- ☐ Schizophrenia
- ☐ Other:

(B) Abstract

Chronic stress affects numerous brain areas involved in memory, emotion, and motivation, such as the hippocampus, amygdala, and prefrontal cortex; it abnormally alters a variety of cellular events, including neuronal morphology and gene expression patterns; and, it can precipitate several maladaptive states, such as depression- and anxiety-like behaviors. Indeed, of all psychiatric disorders, Major Depressive Disorder and Anxiety Disorders are the most common, affecting more than 350 million people worldwide. 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. These sobering results highlight the need for new treatments, which to date have remained scarce and with poorly understood neurobiological mechanisms. In mice and humans, the hippocampus has been implicated in processing positive memories, which have been shown to increase motivation, sociability, mood, and resilience—a psychological phenomenon protecting against stress and preventing various pathological states from precipitating. Additionally, recent methods utilized to chronically stimulate brain circuits, such as deep brain stimulation, have yielded promising therapeutic responses from treatment resistant patients, thus conferring therapeutic value to chronic stimulation protocols that lastingly modulate neural activity. To gain a mechanistic account of how chronically activated circuits supporting positive memories may reprogram neural activity and behaviors, we will test whether or not chronic reactivation of positive memories prior to prolonged stress is sufficient to induce stress resilience. Specifically, we hypothesize that chronically stimulating positive memory bearing hippocampus cells prior to stress can prevent anxiety and depression-like states from precipitating at the cellular level, including preventing neuronal atrophy, as well as the behavioral level, including preventing social impairments, aberrant risk-assessment phenotypes, and anhedonia. We will subsequently perform brain-wide analyses to identify key cellular loci mediating memory's potential prophylactic capacity. Overall, the mission of my lab is to build novel experimental bridges between artificially activated positive memories and animal models of psychiatric disorders—a prospect which dovetails with the Brain & Behavior Research Foundation's mission to understand the neural underpinnings of psychiatric disorders. Indeed, with NARSAD support, I firmly believe my lab will be in a leading position to resolve the neurobiological mechanisms mediating memory's putative therapeutic significance and its role in preventing maladaptive behavior.

(C) Applicant C.V.—NIH Biosketch

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Ramirez, Steve

eRA COMMONS USER NAME (agency login): dvsteve

POSITION TITLE: Post-doc

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Boston University	BA	05/2010	Neuroscience
MIT	PHD	07/2015	Neuroscience
MIT	Postdoctoral Fellow	present	
Harvard	Fellow	present	

A. Personal Statement

The mission of my research is to artificially modulate memories to reverse and prevent psychiatric disease-like states, such as depression- and anxiety-related phenotypes. My previous work identified a defined set of cells active during various memory formation, genetically engineered these cells to respond to light, and optically activated or inhibited these cells while measuring the associated behavioral outputs. These projects focused on mapping out which brain regions were necessary and/or sufficient for memory recall, artificially creating false memories in the rodent brain, modulating the emotional strength associated with specific memories, and leveraging these aforementioned manipulations to reverse depression-like phenotypes. My future projects aim to study the brain-wide cellular mechanisms that confer therapeutic or deleterious value to artificially activated positive and negative memories in mice. To compliment these in vivo perturbations, inherent to my aims is a multidisciplinary approach in which I will perform brain-wide analyses utilizing in vivo and ex vivo physiology, as well as in vitro immunohistochemistry to identify key cellular loci and physiological signatures mediating memory's putative antidepressant and anxiolytic effects. Fittingly, my long-term career goal is answering the following fundamental question in neurobiology: what is the cellular and circuit-level basis for memory and, once artificially activated in the context of stress-induced pathologies, what is the ensuing therapeutic map of dynamic cellular responses? My working hypothesis is that activated positive or negative memories can lastingly reverse or mimic a trifecta of stress-induced pathologies, including abnormal cellular phenotypes such as dendritic atrophy, circuit-wide malfunctioning such as irregular gene expression patterns, and psychiatric-disease like states such as anehdonia. My research will help build an experimental bridge between memory processes in the brain and animal models of psychiatric disorders.

My substantial training in neuroscience and teaching has provided me with the comfort and leadership necessary to successfully organize and execute my proposed projects. To that end, my areas of expertise include rodent molecular and behavioral neuroscience, opto- and pharmacogenetics, immunohistochemistry and pharmacology. For instance, in Howard Eichenbaum's lab at Boston University, we performed in vivo single-unit recordings in awake behaving animals to study how hippocampus cells represent the temporal dimension during various behaviors, which contributed to the discovery of "time cells" published in Neuron. In Susumu Tonegawa's lab at MIT, we created the aforementioned genetic tagging system that has enabled numerous projects centered on testing a variety of exciting hypotheses regarding memory formation and retrieval—each of the four ensuing discoveries by my team was published in Nature or Science. Thus, I firmly believe my lab at Harvard will be in a leading position to resolve the neurobiological mechanisms mediating memory's therapeutic significance and its role in generating maladaptive behavior.

1. Ramirez S, Liu X, MacDonald CJ, Moffa A, Zhou J, Redondo RL, Tonegawa S. Activating positive memory engrams suppresses depression-like behaviour. Nature. 2015 Jun 18;522(7556):335-9. PubMed PMID: 26085274.
2. Redondo RL, Kim J, Arons AL, Ramirez S, Liu X, Tonegawa S. Bidirectional switch of the valence associated with a hippocampal contextual memory engram. Nature. 2014 Sep 18;513(7518):426-30. PubMed PMID: 25162525; PubMed Central PMCID: PMC4169316.
3. Ramirez S, Liu X, Lin PA, Suh J, Pignatelli M, Redondo RL, Ryan TJ, Tonegawa S. Creating a false memory in the hippocampus. Science. 2013 Jul 26;341(6144):387-91. PubMed PMID: 23888038.
4. Liu X, Ramirez S, Pang PT, Puryear CB, Govindarajan A, Deisseroth K, Tonegawa S. Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature. 2012 Mar 22;484(7394):381-5. PubMed PMID: 22441246; PubMed Central PMCID: PMC3331914.

B. Positions and Honors

Positions and Employment

2007 - 2010	Teaching Assistant, Boston University
2010 - 2014	Teaching Assistant, MIT
2014 - 2014	Visiting Lecturer of Neurosience, Tufts University
2015 -	Post-doc, MIT
2015 -	Junior Fellow, Harvard University

Other Experience and Professional Memberships

2010 -	Member, Molecular and Cellular Cognition Society
2010 -	Member, Society for Neuroscience

Honors

2012	Best Abstract, MIT
2012	Angus MacDonald Award for Excellence in Undergraduate Teaching, MIT
2013	Walle Nauta Award for Continued Dedication to Teaching, MIT
2013	World's Top 35 Innovators Under the Age of 35, Technology Review
2013	Top 10 discoveries of 2013, La Recherche
2013	Speaker, TED
2014	World's Top 30 Thinkers Under the Age of 30, Pacific Standard Magazine
2014	Speaker, TEDx
2014	American Ingenuity Award, in the area of the Natural Sciences, Smithsonian Magazine
2014	Top 10 Breakthroughs of the year, Science Magazine
2015	Office of the Dean Graduate Education Diversity Fellowship, MIT
2015	Breakthrough Explorer, National Geographic
2015	30 Innovators Under the Age of 30 , Forbes
2015	Top 10 Bright Young Minds, Science News

C. Contribution to Science

1. My early publications identified a key node in the brain sufficient for activating discrete memories. Recent studies had indicated that defined populations of neurons were cellular correlates of a specific memory trace, or "engram". Previously, other groups had found that selective ablation or inhibition of such neuronal populations erased memory responses, indicating that these cells are necessary for memory expression. However, to demonstrate that a cell population is the cellular basis of a specific engram, it was crucial to conduct a sufficiency experiment to show that direct activation of such a population is capable of inducing the associated behavioral output. I was co-first author on these studies identifying a subset of cells in the hippocampus that processed a specific engram and that were sufficient to activate memory recall.

a. Ramirez S, Tonegawa S, Liu X. Identification and optogenetic manipulation of memory engrams in the hippocampus. Front Behav Neurosci. 2013;7:226. PubMed PMID: 24478647; PubMed Central PMCID: PMC3894458.

b. Liu X, Ramirez S, Pang PT, Puryear CB, Govindarajan A, Deisseroth K, Tonegawa S. Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature. 2012 Mar 22;484(7394):381-5. PubMed PMID: 22441246; PubMed Central PMCID: PMC3331914.

2. We next sought to experimentally demonstrate the malleability of memory by creating a false memory in mice. We accomplished this by optogenetically manipulating engram–bearing cells in the hippocampus to artificially update a neutral memory with aversive information. Importantly, the recall of this false memory was context-specific and activated similar downstream regions engaged during natural memory recall. Thus, our data demonstrated that it is possible to artificially alter the contents of an internally represented and behaviorally expressed memory. I was co-first author on these studies, which provided the conceptual and experimental basis for my more recent experiments seeking to modulate memories in the context of psychiatric disease-like states.

a. Liu X, Ramirez S, Tonegawa S. Inception of a false memory by optogenetic manipulation of a hippocampal memory engram. Philos Trans R Soc Lond B Biol Sci. 2014 Jan 5;369(1633):20130142. PubMed PMID: 24298144; PubMed Central PMCID: PMC3843874.

b. Liu X, Ramirez S, Redondo RL, Tonegawa S. Identification and Manipulation of Memory Engram Cells. Cold Spring Harb Symp Quant Biol. 2014;79:59-65. PubMed PMID: 25637263.

c. Ramirez S, Liu X, Lin PA, Suh J, Pignatelli M, Redondo RL, Ryan TJ, Tonegawa S. Creating a false memory in the hippocampus. Science. 2013 Jul 26;341(6144):387-91. PubMed PMID: 23888038.

3. I recently led research seeking to bridge artificially activated memories and animal models of psychiatric disorders. Chronic stress is a potent diathesis for abnormal gene, cellular, and systems-level processing in the brain and is capable of precipitating depression- and anxiety-like states. 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. As first author or co-author on these studies, our work first showed that we could switch the valence driven by a defined set of memory-bearing hippocampus cells from negative to positive, and vice versa. This work provides the basis for my lab's future experiments seeking to attenuate the emotionally salient components of PTSD-like states, for instance. We then went on to show that optogenetically reactivating positive memories was sufficient to acutely suppress depression-like behaviors and, when chronically activated, were also sufficient to lastingly alleviate such behaviors as well as to promote neurogenesis. This study in particular provides the experimental basis for my currently proposed aims.

a. Tonegawa S, Liu X, Ramirez S, Redondo R. Memory Engram Cells Have Come of Age. Neuron. 2015 Sep 2;87(5):918-31. PubMed PMID: 26335640.

b. Ramirez S, Liu X, MacDonald CJ, Moffa A, Zhou J, Redondo RL, Tonegawa S. Activating positive memory engrams suppresses depression-like behaviour. Nature. 2015 Jun 18;522(7556):335-9. PubMed PMID: 26085274.

c. Redondo RL, Kim J, Arons AL, Ramirez S, Liu X, Tonegawa S. Bidirectional switch of the valence associated with a hippocampal contextual memory engram. Nature. 2014 Sep 18;513(7518):426-30. PubMed PMID: 25162525; PubMed Central PMCID: PMC4169316.

http://www.ncbi.nlm.nih.gov/sites/myncbi/1X__dnnzMk3Au/bibliography/49268422/public/?sort=date&direction= descending

D. Research Support

Ongoing Research Support

Harvard Society of Fellows
Salary support for this project.

Completed Research Support

T32 GM007484-36
SUR, MRIGANKA (PI)
07/01/77-06/30/17
Integrative Neuronal Systems
The goal of this grant was to provide support throughout my graduate training in systems-level neuroscience.
Role: TA

T32 MH074249-06
Bear, Mark F (PI)
07/01/05-06/30/17
Training Program in the Neurobiology of Learning and Memory
The goal of this training grant was to provide support throughout the years leading up to my graduate thesis centered on the circuit-wide mechanisms supporting learning and memory.
Role: TA

T32 GM007484-35
SUR, MRIGANKA (PI)
07/01/77-06/30/12
Integrative Neuronal Systems
The goal of this training grant was to support the initial years of my graduate training in integrative neuronal systems.
Role: TA

T32 GM007484-34
SUR, MRIGANKA (PI)
07/01/77-06/30/12
Integrative Neuronal Systems
The goal of this training grant was to support the initial years of my graduate training in integrative neuronal systems.
Role: TA

(D) Proposal

A sparse population of neurons distributed throughout the brain encodes a specific memory[1]; these cells can be genetically engineered for subsequent artificial manipulation, including in areas such as the hippocampus (HPC), which is pivotal for processing memories of personally experienced events[2-3]. Recently, our work has demonstrated that artificially stimulating cells in the dorsal dentate gyrus subregion of the HPC that previously were active during learning are sufficient for the behavioral expression of negative, neutral, and positive memory recall[4]. These cells also undergo plasticity-related changes during learning and are necessary for memory recall, thus confirming their mnemonic nature[4-5]. We achieved this by developing an optogenetic system in which cells that are active specifically during memory formation are labeled with the light-sensitive ion channel channelrhodopsin-2 (ChR2), thus conferring activity dependent labeling of, and optical control over, memory bearing cells. These findings also raise the intriguing possibility of optogenetically activating memories in the context of stress-induced maladaptive states.

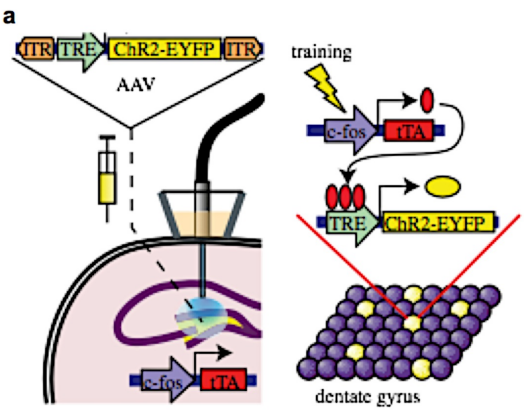

Figure 1. Genetically engineering hippocampus cells active during learning to express ChR2. **(A)** A mouse is injected with a virus cocktail consisting of c-fos-tTA and TRE-ChR2 into the hippocampus, followed by optic fiber implants. When off Dox, the formation of a memory induces the expression of tTA, which binds to TRE and drives the expression of ChR2, thereby labeling a population of activated cells (yellow). (Modified from Liu and Ramirez et al. 2012)

Interestingly, a myriad of anatomical and behavioral studies have suggested that the HPC can be structurally and functionally divided along its dorsal-ventral axis, and that each division differentially responds to stress[6]. For instance, lesions of the ventral hippocampus (vHPC), but not dorsal HPC, impair stress responses and emotional memories[6]. vHPC lesions also modulate behavior in traditional assays of anxiety- and depression-like states, including the open field test (OFT), often used to measure an animal's anxiety levels given rodents' innate tendency to avoid open spaces (i.e. the center of the arena), the sucrose preference test (SPT), often used as a measure of reward-seeking, or hedonic, behavior, and a variety of social interaction assays[7]. Chronic stress increases anxiety-like behavior by decreasing the number of center crossing in the OFT, while increasing depression-like behaviors by diminishing preference for sucrose in the SPT as well as diminishing the total number of interactions a subject will have with a novel subject in a resident intruder test (RIT). These lines of data point to vHPC as an ideal candidate to activate positive memories in the context of stress.

Moreover, while ample therapeutic strategies exist for reversing cognitive maladies, preventative strategies, such as generating stress resilience, remain limited and without well understood biological substrates[8]. Promisingly, it has been proposed that prolonged states of positive affect can promote resilience—a psychological phenomenon protecting against stress and preventing various pathological states from precipitating. Additionally, recent methods utilized to chronically stimulate brain circuits, such as deep brain stimulation, have yielded promising responses from treatment resistant patients, thus conferring therapeutic value to chronic stimulation protocols that modulate neural activity[9]. Thus, to gain a mechanistic account of how chronically activated endogenous processes may reprogram neural activity and behavior, we will test if chronic reactivation of positive memories *prior* to stress is sufficient to induce stress resilience by *preventing* maladaptive phenotypes from precipitating at both the neuronal and behavioral level.

To that end, with NARSAD support, I will begin by targeting the ventral dentate gyrus (vDG) subregion of the HPC and by utilizing our recently developed genetic system, which leverages the activity-dependent nature of the c-Fos promoter and couples it to the tetracycline transactivator (tTA), which, when activated as a result of neural activity, binds to the tetracycline response element (TRE) and thereby promotes transcription of the light-sensitive ion channel channelrhodospin-2 (ChR2) in a doxycycline (Dox)-dependent manner (**Figure 1**). When Dox is removed from an animals' diet, neural activity leads to c-Fos-promoter-driven ChR2 expression in a defined set of HPC cells. When Dox is present, this process is inhibited, thus providing the ability to open and close windows for activity-dependent labeling of cells specifically active during positive memory formation.

Wildtype male and female mice (2-3 months of age) will be injected in vDG with a virus cocktail consisting of c-Fos-tTA and TRE-ChR2-mCherry, or TRE-mCherry as a non-light-sensitive control, which enables us to tag cells active during positive memory formation (**Figure 2**). After recovery, the experimental groups will be taken off Dox to open a window of activity-dependent labeling and subjected to a positive

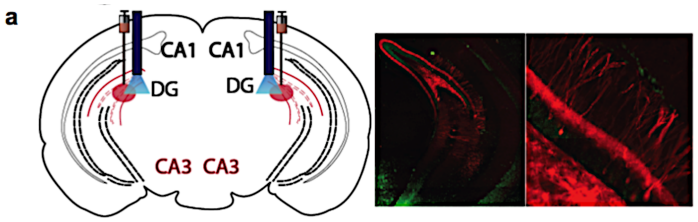

Figure 3. Labeling memory bearing cells in ventral dentate gyrus.
(A) A virus cocktail consisting of c-fos-tTA and TRE-ChR2-mCherry was injected into the ventral hippocampus DG subregion to label cells active during positive memory formation, after which robust ChR2-mCherry expression is observed (right).

experience (i.e. 30 minute exposure to a female conspecific for males; 30 minute exposure to 10 grams of male soiled bedding for females) or a neutral experience as a control (i.e. 30 minute exposure to a novel context)[3-5]. Crucially, the NARSAD award will be utilized to purchase and assemble the subsequent optogenetic and behavioral assays, as well the software necessary to analyze these data during the two-year award period.

Once back on Dox to close the window of activity-dependent labeling of vDG cells, all groups will receive 15 minutes of light stimulation, twice a day for 5 days in their home cages, which is an efficacious protocol for inducing lasting changes in neuronal and behavioral phenotypes[4] (**Figure 3**). All subjects will then undergo chronic immobilization stress (CIS)—a well-validated severe stressor capable of precipitating anxiety and depression-like phenotypes and consists of placing a subject in a restrainer for 2 hours a day for 10 days[4,7,8]. To determine pre-stress behavioral baselines, a control group expressing TRE-mCherry in positive memory bearing vDG cells will receive the same chronic stimulation protocol but will not undergo CIS; to determine post-stress behavioral baselines, a control group expressing TRE-mCherry will receive the same chronic stimulation protocol and will then undergo CIS. Finally, all groups will undergo the RIT, SPT, and OFT, but without optical stimulation to test for lasting positive memory-induced resilience.

To measure hedonic behavior in the SPT, an operant chamber equipped with photolickometers placed on two separate corners will be used to count the number of licks made by water-restricted mice[7,10]. Each photolickometer will contain a lick spout with direct access to 2% sucrose water solution or water alone. In this behavior, unstressed animals prefer sucrose ~80% of the time over water alone, while stressed subjects show approximately a 50/50 preference. To measure changes in sociability, the RIT will occur for 5 minutes in a single day[10]. While in the homecage, a test mouse will be exposed to an intruder juvenile mouse, and a social interaction is defined as any period of time in which the former explores the latter (e.g. sniffing, active contact with the stranger's snout, flank, or anogenital area). In this behavior, unstressed animals show robust social interactions with the intruder animal while stressed subjects show a dramatic impairment in such behavior. To measure anxiety in the OFT, an open metal chamber with transparent plastic walls will be used for 5 minute sessions—unstressed animals explore the center of the chamber considerably more than stressed subjects[5].

If chronic stimulation of positive memories is sufficient to prevent the effects of chronic stress, then we predict a *lack* of stress-induced decreases in sucrose preference, social interactions, and open field center crossings. Additionally, as chronic stress often induces neuronal atrophy and aberrant neuronal activity in areas such as hippocampus subregion CA3 and medial prefrontal cortex[7,10], we will perform a brain-wide spine density analysis and, accordingly, predict that chronically activating positive memories will prevent dendritic spine decreases in numerous areas known to be modulated by stress[8]. As an alternative, preventing stress effects may require longer stimulation protocols; if so, we will extend the stimulation period and duration for up to 14 days, which is when memories begin to become HPC-independent[2,5].

Our proposed experiments provide an innovative application of theoretical research concepts by viewing artificially activated memories as putative anxiolytics and antidepressants, as well as methodological approaches with a system to label cells in an activity-dependent manner with light-sensitive effectors. As a new principle investigator, I am excited by the prospect of leveraging a NARSAD award to jumpstart my lab and test such hypotheses to bridge artificially activated memories and psychiatric disorders. My expertise in cellular and behavioral neuroscience provides me with the comfort and leadership necessary to organize and execute these projects. Indeed, I firmly believe my lab at Harvard will be in a leading position to resolve the neurobiological mechanisms mediating memory's putative therapeutic significance and its role in preventing maladaptive states.

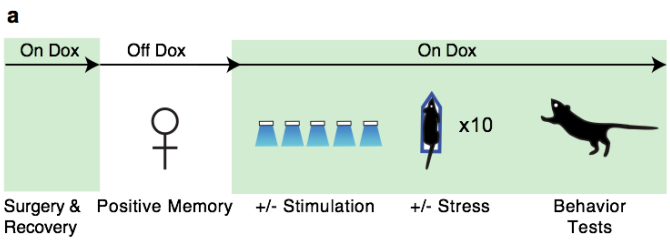

Figure 3. Behavioral schedule for chronic positive memory stimulation prior to stress
(A) Experimental animals will have a virus cocktail consisting of c-fos-tTA and TRE-ChR2-mCherry into the ventral hippocampus CA1 subregion to label positive memory bearing cells while off dox. Next, the positive memory group will receive 5 days of memory reactivation followed by a chronic stress protocol and subsequent behvioral testing.

(E) Research Training/Career Plans

A NARSAD award would serve as a launching pad for my long-term career goal of tackling fundamental questions in neuroscience by building causal bridges across the disciplines of memory and psychiatric disorders. Indeed, my immediate and long-term career goals are to perform rigorous neuroscience research across these two fields and to mentor scientists throughout. 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 terms of recently completed research training, I earned my Ph.D. in neuroscience from MIT in 2015—during this time, I mentored undergraduates and technicians, as well as guided graduate students and post-doctoral fellows in terms of techniques and experimental design. After acquiring expertise in each of the subsequent areas during my graduate tenure, I provided training to numerous members of my graduate lab in *in vivo* optogenetics, behavioral assays, immunohistochemistry, stereotaxic surgeries, animal husbandry, and single-cell data analyses. Together, we went on to develop a system to identify and manipulate cells processing positive neutral, and negative memories. My roles also included leading frequent group meetings, designing experiments, conflict management, as well as building and maintaining novel cellular and behavioral assays. Thus, I rapidly became cognizant of the group dynamics needed to carry out a set of experiments effectively and efficiently; as such, I am confident in my abilities to enable exceptional neuroscience and to provide hands-on mentorship to trainees in my lab.

I recently joined Harvard as a Junior Fellow of the Society of Fellows—a program that provides the opportunity to lead independent research in any department of the University, free from formal requirements. Soon after, I was recruited by Harvard's Center for Brain Science, where I am currently a Principle Investigator and in the process of building an independent research group. To that end, I have already begun meeting with junior and senior faculty to setup collaborations, to share both equipment and reagents, and to schedule open lab meetings so as to optimize the process of troubleshooting and executing experiments accordingly. Indeed, to facilitate my future career plans of joining as tenure-track faculty, I have made extensive arrangements with various faculty and staff to provide the support and feedback necessary to establish my research program while maintaing my intellectual independence. More specifically, to realize my research career objective of artificially commandeering neuronal circuits processing memories to thereafter exploit their potential ability of rehabilitating and preventing impaired cognitive processes, I have had ample interactions with the students, staff, and professors at the Department of Molecular and Cellular Biology and the Center for Brain Science, including Drs. Joshua Sanes, Venki Murthy, Nao Uchida, and David Cox—all of whom have agreed to provide ample support with grant writing, hiring personnel, sharing equipment, and in meeting individually regularly to assess my personal development and the progression of my proposed project. Finally, to further integrate myself as an active member of Harvard's community in the coming years, I welcome members within our building to join our lab meetings to provide critical feedback; conversely, I encourage members of my lab to join in the aforementioned professors' lab meetings, as well as to present in departmental seminars, to diversify their experimental and conceptual skillsets. Fittingly, a NARSAD award would fortify my academic trajectory by imparting the independence and support necessary to launch a fruitful research career, which excitingly would begin by testing if chronically activated positive memories confer lasting stress resilience.

(F) Budget
2-year grant: $35,000 per year requested

	Item	Price (U.S. $)	Justifaction
Year 1	Dual Arm Stereotax	12,000	To perform surgeries on mice and inject our virus cocktail that genetically engineers cells active during positive memory formation to become light sensitive.
	Blue Laser x 2	5,000	To perform optogenetic experiments in which blue light will be delivered to cells harboring positive memories.
	Behavior Chambers x 3	12,000	These versatile chambers will be used for a variety of behavioral measures, including social interactions, reward conditioning to elicit positive memories, depression-like behaviors, and overall locomotion levels.
	Pulse Generator	3,000	This piece of equipment will be utilized to deliver precise optogenetic stimulation at any given frequency.
	Rotary Joints	3,000	This piece of equipment enables an animal to be tethered to an optic fiber through which light wil be delivered while also being able to move freely (e.g. during social exploration) without any hindrances.
Year 2	Dual Arm Stereotax	12,000	See above
	Blue Laser x 2	6,000	See above
	Pulse Generator	4,000	See above
	Antibodies	3,000	Antibodies are needed to stain for ChR2-mCherry and to visualize brain cells, as well as to test for virus efficacy and measure accuracy of surgical injections.
	Behavior Software	6,000	Software wil be used to automatically score behaviors in an a high-throughput, unbiased, and reliable manner.
	Custom Behavior Chambers	4,000	With the help of in-house engineers at Harvard's Center for Brain Science, we will build these chambers that traditionally are used to measure an animal's overall anxiety levels.

The unique contribution of this grant to my research activities is that it directly enables assembling and utilizing the experimental tools needed to test the hypothesis that chronically activated positive memories are sufficient to induce stress resileience. All equpiment and software will be dedicated specifically to test the proposed hypothesis throughout the award period. Importantly, as a current Junior Fellow at Harvard and a new principle investigator, these resources are not currently available to my lab and, as such, their use will be crucial to enable each line of investigation in this proposal. The impact of this grant on institutional support includes full utilization of facilities for histological and behavioral testing, as well as enabling collaborations and equipment sharing with Harvard's faculty. Receipt of this grant will not result in duplication of funding or reduction of institutional support.

Pending Research support
Source: National Institutes of Health, Director's Early Independence Award
Project title: Artificially modulating memories to alleviate psychiatric-disease like states
Dates of project period: 09/2016 – 08/2021
Amount: $1,250,000
Status: *Application under review*