Summary: Chronic stress affects numerous brain areas involved in memory, emotion, and motivation, such as the hippocampus (HPC) and various cortical areas; 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. Yet, the neural circuitry sufficient to mitigate or prevent such pathological phenotypes is unclear. In mice and humans, the HPC has been implicated in processing positive memories and in modulating stress-related states. To that end, in this proposal, I aim to build a novel experimental bridge between artificially activated positive memories and animal models of psychiatric disorders. The research plan representative of the kinds of projects undertaken in my lab contains three Goals: **Goal 1**) acutely activate memories after chronic stress and measure the underlying neuronal responses in real-time; **Goal 2**) deconstruct memories into their component axonal terminals and physiologically correct each output after chronic stress to thereby correct maladaptive behaviors; **Goal 3**) chronically activate positive memories prior to stress to induce resilience and characterize the underlying neuronal landscape in vivo.

Significance: My proposed research aims to provide a neurobiological framework for artificially activating memories to bi-directionally modulate stress-induced maladaptive states. **Goal 1** offers an acute method to suppress stress-related pathologies as well as a novel means to precipitate and characterize them at the cellular and behavioral levels. **Goal 2** offers to deconstruct memories into their component terminals before and after stress, followed by physiologically correcting each terminal post-stress and testing for its capacity to reverse independent features of stress-induced behaviors, including reward-related, anxiety-like, and social impairments. **Goal 3** aims to measure whether or not chronic activation of positive memories *prior* to stress induces neuronal and behavioral resilience, thus testing for memory's potential prophylactic capacity, which, if existent, will enable a brain-wide in vitro and in vivo search for key cellular and physiological loci mediating the protective effects. Overall, the significance of these proposed experiments is that they seek to commandeer the brain's endogenous plasticity mechanisms by means of memory activation to demonstrate and resolve its therapeutic potential as well as its role in regulating maladaptive circuit functioning and behavior.

Background:

Immediate early gene activity and memories

Previous genetic, electrophysiological, and behavioral studies have suggested that a sparse population of neurons distributed throughout the brain encodes a specific memory (Guzowski, 2002; Tonegawa et al. 2015). These neurons can be tagged during learning for subsequent identification and manipulation (Han et al. 2009). The hippocampus (HPC) in particular is pivotal for the encoding, storage, and retrieval of personally experienced, or episodic, memories (Eichenbaum, 2004). Recently, our work has demonstrated that HPC cells in the dentate gyrus (DG) subregion of the HPC that previously expressed the immediate early gene (IEG) c-Fos during learning are sufficient to activate the neuronal and behavioral expression of negative, neutral, and positive memory recall (Liu et al. 2012; Ramirez et al. 2013; Redondo et al. 2014). These cells also undergo plasticity-related changes during learning (Ryan et al. 2015) and are necessary for the behavioral expression of memory recall (Denny et al. 2014; Tanaka et al. 2014), thus corroborating their mnemonic nature

Dorsal vs. ventral HPC in anxiety and depression-like states

A myriad of anatomical, lesion, and behavioral studies have suggested that the HPC can be structurally and functionally segregated along its dorsal-ventral axis, and that each division differentially responds to stress (Fanselow and Dong 2010). For instance, lesions of the ventral, but not dorsal, HPC impair stress responses, and lesions of the dorsal or ventral HPC differentially affect spatial or emotional memories, respectively (Moser and Moser, 1995). Ventral HPC lesions modulate behavior in traditional assays of anxiety- and depression-like states, including the open field test, often used to measure an animal's tendency to avoid open spaces (i.e. the center of the arena), the sucrose preference test, often used to measure hedonic-like behavior, and social interaction assays (McEwen et al. 2015). Specifically, chronic stress increases anxiety-like behavior by decreasing the number of center crossing in the open field, while increasing depression-like behavior by diminishing preference for sucrose and the total number of interactions a subject will have with a novel subject (Nestler and Hyman, 2010). These lines of data point to the ventral HPC as an ideal candidate to activate mnemonic processes in the context of such stress-induced states.

Activating memories to alter behavioral states

To activate memories in healthy and chronically stressed rodents, we recently built on an activity-dependent and inducible system to tag HPC neurons active during memory formation (Reijmers et al. 2007).

Our system 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 cells. When Dox is present, this process is inhibited, thus providing the ability to open and close windows for activity-dependent labeling and optical manipulation of HPC neurons (Liu et al. 2012).

Promisingly, our previous work demonstrated that optogenetically reactivating dorsal HPC-mediated positive memories in chronically stressed mice successfully reversed motivational impairments and anhedonia, as assayed by the tail suspension test and sucrose preference test (**Figure 2**). Our manipulations, however, were performed *post*-stress, thus leaving memory's putative prophylactic properties untested, as well as the underlying neuronal landscape unknown. Also, they did not suppress anxiety-related measures, conceivably as a result of targeting dorsal and not ventral HPC. As such, our proposed representative experiments focus on ventral HPC and its outputs given its role in emotional, social, and anxiety-like behaviors (Kheirbek et al. 2013).

Goal 1) Test for antidepressant and anxiolytic effects of ventral HPC-mediated memory modulation
Rationale: To understand how artificially activated memories may modulate stress-induced maladaptive states, we must first identify memory bearing cells in ventral HPC (vHPC) and engineer them to express ChR2, followed by optically activating this defined set of HPC cells and measuring their capacity to regulate neuronal and behavioral states. The timeline for all subsequent experiments is outlined in **Table 1**.
Experimental Design: Wildtype male and female C57BL/6J mice (2-3 months of age) will be injected in vHPC with a virus cocktail of AAV_9-c-Fos-tTA and AAV_9-TRE-ChR2-mCherry, which enables us to tag cells active during positive or negative memory formation. Control mice will be injected with AAV_9-TRE-mCherry (**Figure 3**). We will target ventral CA1 (vCA1) given its robust outputs to various stress-modulated areas. Female estrus stages will be monitored in all experiments for potential cycle effects. These experiments test the hypothesis that activated positive or negative memory vCA1 cells bi-directionally control stress-induced states.
(A) Identifying positive or negative memories in vCA1. Male and female mice will be injected with the aforementioned virus cocktail in vCA1, followed by a 10-day recovery period. A control group will remain on Dox throughout to confirm inducibility. After recovery, the experimental groups will be taken off Dox to open a window of activity-dependent labeling and subjected either to a positive experience (i.e. 30-minute exposure to a female conspecific for males; 30-minute exposure to 10 grams of male soiled bedding for females; Redondo et al. 2014; Moncho-Bogani et al., 2005) or a negative experience (i.e. 3-shock fear conditioning session; Liu et al. 2012; **Figure 4a**). Once back on Dox for 24 hours, subjects will be sacrificed and prepared for histology by processing HPC slices and performing antibody staining against ChR2-mCherry to confirm activity-dependent expression, functionality, and injection site accuracy.
(B) Optically reactivate vHPC-mediated memories during depression- and anxiety-related assays. To test the hypothesis that vHPC-mediated memories can modulate a separate set of stress-induced behaviors compared to our previous results in the dorsal HPC, mice will have positive or negative memory bearing vCA1 cells labeled with ChR2-mCherry or mCherry only as a control, after which all groups will be immediately placed back on Dox to prevent further labeling as described in Aim1A (**Figure 4a**). The following day, the positive memory group will be exposed to a chronic immobilization stress procedure, which is a well-characterized severe stressor capable of precipitating depression- and anxiety-like phenotypes (Lim et al. 2012). Another control group will be kept unstressed, as well as the negative memory group—the latter will be kept unstressed because my recent findings suggest that a behavioral floor effect is present in stressed animals that also undergo negative memory stimulation (Ramirez et al. 2015). To test if activated positive memories can reverse the effects of stress on hedonic activity and sociability, mice will undergo a sucrose preference and resident intruder test (SPT, RIT), while the open field test (OFT) will be used to measure anxiety-like behavior. To test if activated negative memories can elicit anhedonia, social impairments, and anxiety-like behavior, the unstressed vCA1 negative memory group will receive optical stimulation during the same behavioral assays. Promisingly, my preliminary data suggest that activated vCA1 positive or negative memory HPC cells are sufficient to decrease or increase anxiety-like behaviors, respectively, in unstressed mice, (**Figure 4b**).

For the SPT, an operant chamber equipped with photolickometers placed on two separate corners of the chamber will be used to count the number of licks made by the water-restricted mice at lick spouts with direct access to 2% sucrose water solution or water alone. The test lasts 30 minutes and is divided into a 15-minute light-off and 15-minute light-on epoch (Ramirez et al. 2015). The RIT will occur for 5 minutes, once a day for 2 days, with light-off or light-on epochs occurring for the entirety of the session and counter-balanced

between days. While in the homecage, a test mouse will be exposed to an intruder juvenile mouse (Felix-Ortiz 2013, 2014); an 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). For the OFT, an open metal chamber with transparent, plastic walls will be used for 9 minute sessions with light stimulation given during minutes 3-5 inclusive (Tye et al. 2011). If vCA1 stimulations fail to produce effects, we will activate ventral dentate gyrus (vDG) or CA3 (vCA3) (**Figure 5**) cells with the same parameters. Alternative stress protocols will be used for males and females if CIS fails to precipitate changes in the proposed behaviors, including chronic social defeat stress, which is known to be robust in males (Berton et al. 2006), and chronic variable stress, which is known to be robust in females (Mineur et al. 2006).

(C) Perform brain-wide analyses to identify and characterize downstream loci activated by vHPC stimulations. To identify neural loci mediating the positive or negative memory-induced changes in behaviors, a brain-wide immediately early gene assessment will be performed after the chronic stress protocol. Four groups will be utilized: two groups will contain vCA1 ChR2-mCherry-positive cells labeled by either a positive or negative experience, which will then receive light-stimulation using the same light delivery parameters described in **Goal 1A**. The other two groups will contain mCherry-only cells in vCA1 labeled by a positive or negative experiences and serve as negative controls. All groups will be sacrificed 90 minutes after light stimulation—the time point when c-Fos protein expression is maximal as a marker of recent neural activity (Guzowski, 2002)—and brains will be sectioned, fixed, and undergo antibody staining against c-Fos, followed by confocal imaging. c-Fos-positive cells will be quantified per brain region and compared across groups; candidate areas for analyses will be chosen based on previous research implicating these areas in mediating depression- and anxiety-like behaviors (e.g. amygdala, prefrontal cortex; Nestler and Hyman 2010). Next, four groups will be prepared as before, except regions identified as significantly active in response to memory reactivation will be injected with the red-shifted RCaMP6 family of Ca2+ indicators (e.g. AAV2/5-Syn-RCaMP) to permit real-time imaging of Ca2+ transients with a microendoscope across hundreds of cells. Response kinetics and activity dynamics from all groups will be compared during light off and light on epochs in the RIT, SPT, and OFT. I hypothesize that positive memory activation will elicit robust, time-locked activity in various areas associated with reward, including the amygdala and nucleus accumbens; conversely, I predict that negative memory activation will elicit robust time-locked activity in areas associated with aversion, including the central amygdala and habenula.

Relevance to the field: Positive memories in humans have been shown to be inherently rewarding and capable of increasing both mood and sociability (Speer et al. 2014). Moreover, patients with depression show profound impairments in recalling specific positive memories and tend to preferentially recall negative memories (Holmes et al. 2016). **Goal 1** thus aims to directly activate the endogenous processes supporting positive or negative memories to promote or attenuate preference for sucrose, social interactions, and anxiety-like behavior, respectively—thereby revealing a novel intervention for such stress-induced states, as well as a causal method of generating them with negative mnemonic influences known to occur in humans. Such findings would demonstrate that activated memories are sufficient to bi-directionally modulate depression and anxiety-like behaviors, thus enabling a brain-wide search for candidate mechanisms supporting their real-time effects. This search holds the promise of providing the biomedical community with neuronal and physiological markers that can be future interventional and preventative targets to ameliorate the effects of chronic stress.

Goal 2) Test the causal role of vHPC outputs in promoting or reversing stress-related behavior

Rationale: We will study how stress impacts positive or negative memory-bearing vHPC outputs and reverse stress-induced changes at defined axonal terminals to rescue independent features of maladaptive states. Indeed, pathological states, such as anhedonia, social impairments, and anxiety are hallmarks of mood disorders; yet, developing novel interventions targeting individual symptoms has posed a formidable challenge to the biomedical community. These experiments seek to address this challenge by disassembling memories into their component terminals—which route distinct mnemonic information to various stress-modulated target areas—to differentially modulate separable features of psychiatric disease-like states.

Experimental Design: Wildtype male and female mice will be injected in vCA1 with the virus cocktails from **Goal 1**, followed by positive or negative memory-induced labeling of vCA1 terminals in bed nuclei of the stria terminalis (BNST), hypothalamus, or medial prefrontal cortex (mPFC) to test for their functionality—these three regions have emerged as candidates in modulating stress-responses during reward, social, and anxiety-like behaviors, though how vCA1 mnemonic information alters activity in these areas before and after stress, remains untested (Kim et al. 2013; Deisseroth, 2015; Padilla-Coreano et al. 2016). After stress, each target-specific terminal will be physiologically assessed and independently modulated to test if restoring its activity reverses the effects of stress on both neuronal functioning and specific sets of behaviors.

(A) Test the functionality of ChR2-positive vCA1 outputs to BNST, hypothalamus, and mPFC. Four groups of mice will have vCA1 cells processing a positive or negative memory labeled with ChR2-mCherry or mCherry-only in controls as described in **Goal 1B**. Optogenetic stimulation will be delivered for 10 minutes to vCA1 terminals in either the BNST, hypothalamus, or mPFC, based on previously published protocols for terminal-specific activation (Ramirez et al. 2015). Tissue processing will be performed as described in **Goal 1** to measure ChR2-mCherry expression at each projection and light-induced c-Fos expression in each target.
(B) Physiologically assess the impact of chronic stress on memory bearing vCA1 outputs. Previous reports have demonstrated that chronic stress aberrantly increases vCA1 terminal activity in the nucleus accumbens and that long-term depression at this synapse induces antidepressant-like responses (Bagot et al. 2015). A recent study also demonstrated in unstressed animals that vCA1 to mPFC outputs are necessary for anxiety-like behavior (Padilla-Coreano et al. 2016). These studies excitingly provide direct links between monosynaptic vCA1 activity and anxiety as well as depression-like behavior, and here we seek to prove the causal contributions of vCA1 *memory bearing* terminals in regions known to influence anxiety, reward, and social behavior, such as the BNST, hypothalamus, and mPFC, as well as how stress physiologically affects each projection. To that end, mice will be injected with the virus cocktail from **Goal 1B** in vCA1 and will have vCA1 cells processing positive or negative memories labeled with ChR2-mCherry. Before and after chronic stress, brain slices will be prepared for in vitro physiological measurement of terminal-specific responses in BNST, hypothalamus, and mPFC. In the positive memory group, I predict that stress 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, as measured using standard in vitro physiology measures (e.g. paired pulse ratios, AMPAR/NMDAR ratio and rectification index; Ryan et al. 2015), thus guiding the experiments in **Goal 1C.**
(C) Optically correct and map vCA1 outputs processing memories to BNST, hypothalamus, and mPFC after stress. Mice will be injected with the viruses from **Goal 1B** in vCA1 and will have optic fibers implanted over the BNST, hypothalamus, or mPFC to permit terminal-specific optogenetic manipulation. vCA1 cells will then be labeled with a recently developed variant of ChR2, namely, ChIEF, which permits optical stimulation of up to 100hz and has been shown to be capable of inducing long-term potentiation or depression (LTP, LTD) at defined synapses (Nabavi et al. 2014). We have engineered a TRE-ChIEF construct to permit activity-dependent labeling and rapid control of positive and negative memory bearing vCA1 cells. We will use the same timeline of labeling and stress described in **Goal 1B.** To correct the effects of stress at a specific vCA1 projection, animals in which stress induces aberrant increases, or potentiation of, activity at these terminals will undergo an LTD protocol consisting of 1 Hz stimulation for 15 minutes to normalize activity. Conversely, animals in which stress induces decreases, or depression of, activity at each defined terminal will undergo an LTP protocol consisting of 5 bursts of 100 Hz stimulation to restore activity (Nabavi et al. 2014). All groups will have one vCA1 set of projections manipulated and will be tested in the OFT, SPT, and RIT to measure if optically resetting activity at each synapse differentially alleviates stress-induced behavioral changes. Given the suggested role of BNST, hypothalamus, and mPFC in assembling reward, social, and anxiety-related behaviors (Deisseroth et al. 2014), I predict that optically modulating activity in the opposite direction promoted by stress at each unique vCA1 projection will rescue baseline synaptic firing and each corresponding behavior, thereby providing a causal basis for synaptic dysfunctions and their associated behavioral impairments.

Next, to map out the neuronal landscape supporting each putative light-induced change in behavior, separate groups will be prepared in which a combination of retrobeads and retrograde viruses will be used in BNST, hypothalamus, and mPFC, in conjunction with TRE-ChR2-mCherry labeling of vCA1 cells and projections. For instance, it is possible that positive memory bearing vCA1 terminals preferentially synapse onto a subset of BNST cells that themselves preferentially project to areas involved in reward, such as the ventral tegmental area, while separate positive memory bearing vCA1 terminals may target mPFC cells that preferentially project to areas involved in anxiety, such as the amygdala. These experiments will enable a systematic evaluation of the potential topography extant in vCA1 mnemonic-carrying target-specific terminals.
Relevance to the field: Recent physiology studies suggest that vCA1 outputs selectively transmit distinct computations to areas such as the amygdala and nucleus accumbens (Ciocchi et al. 2015). Yet, most vHPC studies to date 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 the BNST, hypothalamus, and mPFC unknown. Notably, in humans, depression and anxiety display tremendous symptom heterogeneity, which remain without effective symptom-specific treatments (Ressler and Mayberg, 2007). Thus, **Goal 2** is relevant to the field because stimulation and mapping of memory-bearing projections to the BNST, hypothalamus, and mPFC may differentially modulate anhedonia, sociability, and anxiety—core symptoms of mood disorders in which positive memories and emotions are impaired (Holmes et

al. 2016). Leveraging independent features of memories to intervene with stress-related states will help resolve the computations performed at defined terminals as well as their causal contributions to maladaptive states.

Goal 3) Determine if chronic activation of vHPC-mediated positive memories induces stress resilience
Rationale: While strategies exist for reversing cognitive and behavioral maladies (Seligman et al. 2006), preventative strategies remain scarce and with few delineated biological mechanisms (Feder et al. 2009). To address this challenge, we 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 characterizing the underlying, brain-wide neuronal mechanisms supporting memory's putative prophylactic capacity.
Experimental Design: We will use wildtype male and female mice, the virus cocktail from **Goal 1**, and chronic stimulation of positive memory HPC cells prior to chronic stress, followed by brain-wide histological and in vivo imaging analyses to search for neurophysiological markers mediating memory-induced changes in behaviors.
(A) Determine the effects of chronic positive memory stimulation on subsequent stress-modulated behaviors.
My previous results suggested that optogenetically activating positive memories for 15-minute sessions twice a day for 5 days was sufficient to reverse the effects that stress had on motivation and hedonic-like behaviors, as well as on neurogenesis, thereby confirming that such a protocol is capable of reprogramming neural circuits and behaviors (Ramirez et al. 2015). Whether or not such stimulation parameters can *prevent* the effects of stress, however, remains untested. vCA1 cells processing a positive memory will be labeled with ChR2-mCherry as outlined in **Goal 1A**. Next, all groups will receive 15 minutes of light stimulation, twice a day for 5 days, followed by CIS, as described in **Goal 1B** (**Figure 6**). To determine upper (i.e. pre-stress) behavioral baselines, a control groups expressing TRE-mCherry in positive memory bearing cells will receive the same chronic stimulation protocol but will not undergo CIS; to determine lower (i.e. post-stress) behavioral baselines, a control group expressing TRE-mCherry will receive the same chronic stimulation protocol and will subsequently undergo CIS. Finally, all groups will undergo the RIT, SPT, and OFT, as in **Goal 1B**, but without optical stimulation, to test for lasting memory-induced resilience. Importantly, preventing stress effects may require longer stimulation protocols; as such, we will extend the stimulation period for up to 14 days, which is when memories begin to become HPC-independent (Tayler et al. 2013).
(B) Identify loci affected by chronic memory activation with histological and novel in vivo imaging analyses.
Here, we 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 Ca2+ transients) observed in areas such as CA3, mPFC, and amygdala (Roozendaal et al. 2009). To test this, after behavioral testing, all groups will be sacrificed and brain slices will be prepared as described in **Goal 1C**. Candidate areas for immediate early gene and spine density analyses will be chosen based on previous work implicating each area in stress responses and behaviors (Deisseroth, 2014). Additionally, my previous work demonstrated that chronically activating positive memories can increase neurogenesis in the HPC (Ramirez et al. 2015). As neurogenesis is necessary for the effects of certain antidepressants and sufficient to reverse the effects of stress in a subset of behaviors (Hill et al. 2015), we will use doublecortin and BrdU staining methods to test if chronically activated positive memories prevent stress-induced decreases in adult-born HPC neurons.

Finally, lead by the histological and spine analyses, we will then 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 Ca2+ transients specifically in cells that were previously active during positive memory formation across a variety of brain areas, such as mPFC and amygdala, and throughout our behavioral schedule. We will record activity in vivo before and after chronic positive memory stimulation both at baseline and during the OFT, SPT, and RIT to note changes in response kinetics and activity dynamics, thereby providing physiological markers for preventative strategies with unprecedented spatial resolution.
Relevance to the field: In humans, it has been proposed that prolonged states of positive affect can promote resilience, which protects against stress and prevents various pathological states from precipitating (Feder et al. 2009; Speer et al. 2014). Moreover, recent methods that chronically stimulate brain circuits, such as deep brain stimulation, have yielded promising responses from treatment resistant patients (Mayberg et al. 2005), thus conferring therapeutic value to chronic stimulation protocols that lastingly modulate neural activity. To gain mechanistic and causal insight into these processes, we predict that chronic activation of positive memories will confer resilience, which will be behaviorally manifested as a *lack* of stress-induced decreases in sucrose preference, social interactions, and open field center crossings, as well as a lack of stress-induced changes in neuronal structure and function. **Goal 3**'s relevance is that it seeks a novel preventative strategy and to identify its associated physiological signatures against dysregulated neuronal, circuit, and behavioral functioning.

a

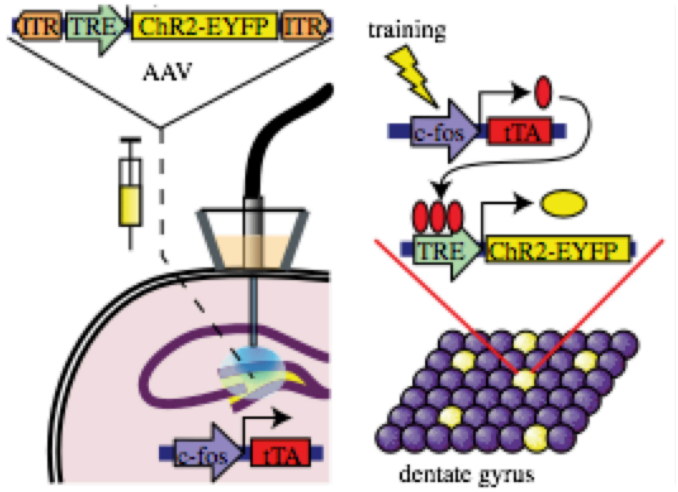

Figure 1. Genetically engineering hippocampus cells active during learning to express ChR2.
(A) A mouse is injected with a virus cocktail consisting of c-fos-tTA and TRE-ChR2 into the hippocampus, followed by optic fiber implants. When off Dox, the formation of a memory induces the expression of tTA,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

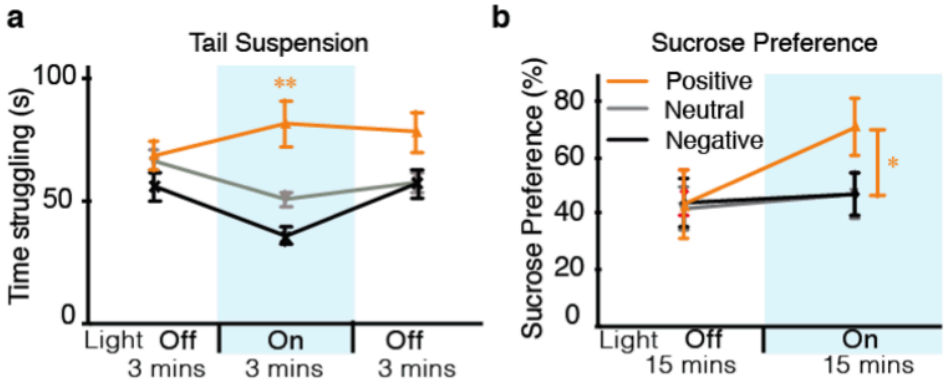

Figure 2. Activating a positive memory rescues depression-like behavior.
(A-B) Optical reactivation of dentate gyrus cells that were previously active during positive (orange), but not neutral (gray) or negative (black) memory formation significantly increases time struggling in the tail suspension test (a) and preference for sucrose (b). (Modified from Ramirez et al. 2015)

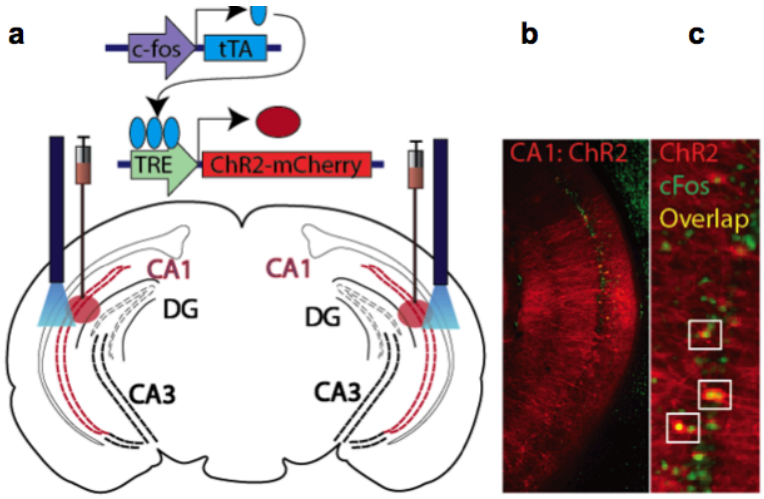

Figure 3. Labeling ventral hippocampus-mediated memories
(A) A virus cocktail consisting of c-fos-tTA and TRE-ChR2-mCherry was injected into the ventral hippocampus CA1 subregion to label cells active during positive memory formation . **(B-C)** Ventral CA1 cells show robust expression of ChR2-mCherry and are functional, as evidenced by light-induced c-fos expression which overlaps with ChR2-mCherry-positive cells (outlined by white squares).

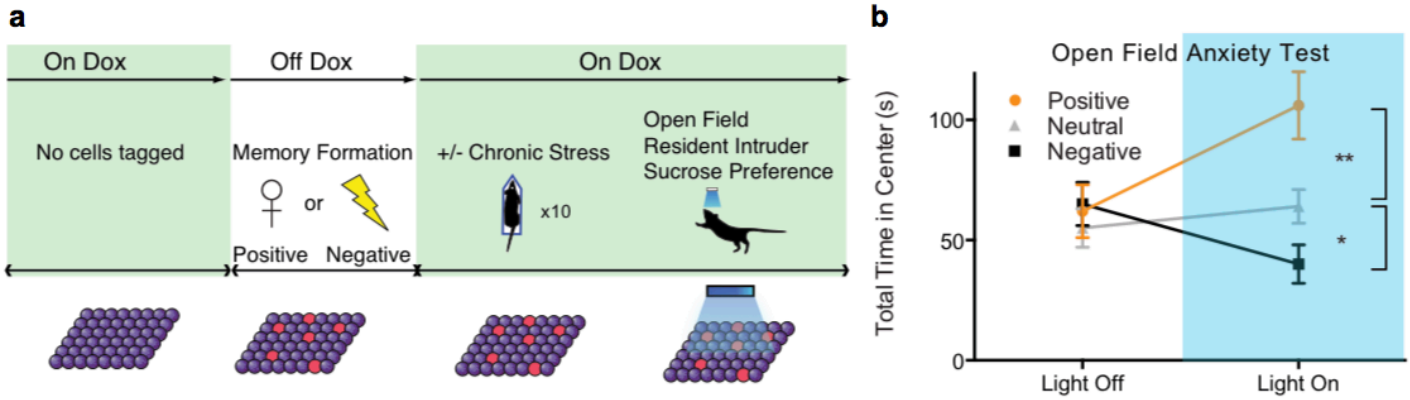

Figure 4. Reactivating vCA1-mediated memories bi-directionaly control anxiety-like behavior
(A) Behavioral schedule. Animals are kept on dox to prevent tagging of active cells with ChR2. Next, they are taken off dox and cells active during the formation of a positive (female symbol) or negative (lightning 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 but without chronic stress. **(B)** vCA1 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 vCA1 cells are sufficient to induce anxiogenic-like behavior.

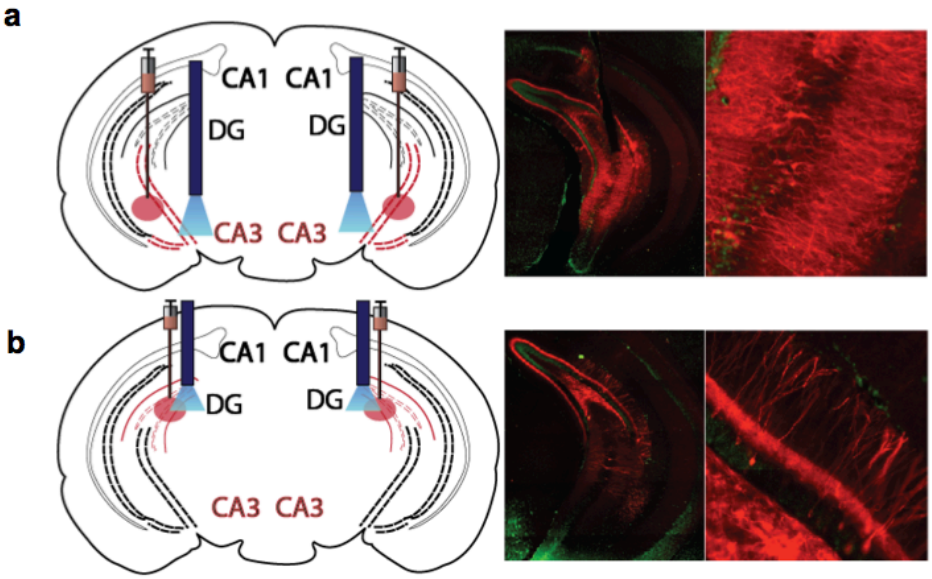

Figure 5. Labeling memory bearing cells in ventral CA3 and DG.
(A-B) A virus cocktail consisting of c-fos-tTA and TRE-ChR2-mCherry was injected into the ventral hippocampus CA3 (a) or DG (b) subregions to label cells active during positive memory formation, after which robust ChR2-mCherry expression is observed (right).

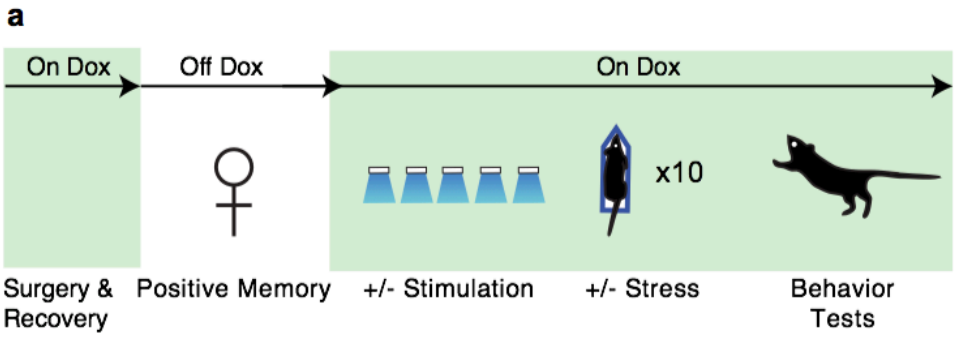

Figure 6. Behavioral schedule for chronic positive memory stimulation prior to stress
(A) Experimental animals will have a virus cocktail consisting of c-fos-tTA and TRE-ChR2-mCherry into the ventral hippocampus CA1 subregion to label positive memory bearing cells while off dox. Next, the positive memory group will receive 5 days of memory reactivation followed by a chronic stress protocol and subsequent behvioral testing.

Table 1

	Year 1	Year 2	Year 3	Year 4	Year 5
Research:					
Aim 1	Order equipment Hire 1 Post-doc Hire 1Technician Recruit 2 undergrads	Hire 1 Post-doc Recruit 1 undergrad	Write Manuscript		
	Characterize c-fos-tTA/TRE-ChR2 expression in vHPC subregions	Perform in vivo calcium imaging experiments			
	Test the effects of activated positive and negative memories on social, sucrose preference, and anxiety behaviors				
Aim 2	Characterize c-fos-tTA/TRE-ChR2 expression in vHPC axonal terminals	Test the effects of terminal-specific manipulations in social, sucrose preference, and anxiety behaviors		Write Manuscript	
Aim 3		Optimize and perform chronic memory stimulation experiments		Perform brain-wide anatomical, histological, and in vivo imaging analyses	Write Manuscript

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