Significance: This research aims to provide a neurobiological framework for modulating memories to alleviate psychiatric disease-like states. More specifically, the neural circuits sufficient to mitigate post-traumatic stress disorder (PTSD) are largely unknown and, to date, nearly every drug-based intervention relies on modulating the same molecular targets discovered in the mid-1900s. These sobering results highlight the need for novel interventions for the cellular and behavioral impairments comprising PTSD and various disorders with which it is highly comorbid, such as depression, which collectively affects more than 300 million people worldwide. To directly tackle this problem, we recently developed a novel genetic strategy for artificially manipulating memories in rodent models of mood and anxiety disorders—a prospect that speaks directly with the McKnight Foundation's mission to prevent and treat disorders of the brain. We discovered a discrete set of hippocampus (HPC) neurons that are sufficient to activate negative, neutral, and positive memories; moreover, silencing their activity inhibits memory recall, thus corroborating their mnemonic nature. The significance of these proposed experiments is to artificially commandeer the brain's endogenous plasticity mechanisms by means of memory activation to demonstrate and resolve its therapeutic potential for alleviating cellular, circuit-level and behavioral abnormalities comprising psychiatric disease-like states in general and PTSD in particular.

Approach: Artificially activating memories to alter behavioral states Previous genetic, physiological, and behavioral studies have suggested that a sparse population of HPC neurons process specific memories. Still, most studies to date intervene with HPC cells by modulating entire sub-regions, despite evidence suggesting that memories recruit only a fraction of HPC cells. To address this issue, our system leverages the activity-dependent nature of the c-Fos promoter and couples it to the tetracycline transactivator (tTA), which, when activated, binds to the tetracycline response element (TRE) and promotes transcription of any downstream sequence, such as the light-sensitive ion channel channelrhodospin-2 (ChR2) or the calcium indicator RCaMP6f, which can be used to monitor cell responses *in vivo*. The capacity of tTA to bind to TRE is controlled in a doxycycline (Dox)-dependent manner (**Figure 1**): when Dox is removed from an animal's diet, neural activity leads to c-Fos-promoter-driven TRE-ChR2 or our newly developed TRE-RCaMP6f 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 tagging of cells. Promisingly, our recent work established that activating positive memories reversed two depression-related behaviors: motivational impairments and anhedonia, as assayed by the tail suspension and sucrose preference test (**Figure 2**).

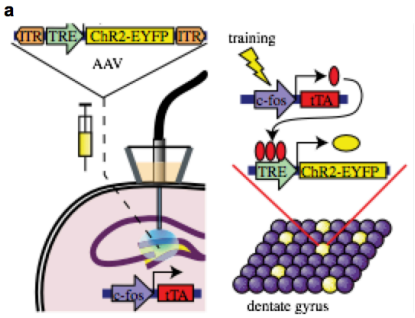

Figure 1. Genetically engineering hippocampus cells active during learning to express ChR2. **(A)** A mouse is injected with a virus cocktail consisting of c-fos-tTA and TRE-ChR2 into the hippocampus, followed by optic fiber implants. When off Dox, the formation of a memory induces the expression of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

Specific Aim 1: Can aversive memories be artificially and permanently suppressed?

Rationale: PTSD is the fourth most common psychiatric disorder and estimates suggest that up to 90% of all people will be exposed to a severe traumatic event during their lifetime, with 10-20% developing PTSD. The neural substrates of fear dysregulation are remarkably well conserved, yet the neural landscape sufficient to suppress maladaptive fear responses remains poorly understood. To that end, we will first tag HPC cells that were active during fear memory formation (Fear Group) or positive memory formation (Positive Group) with TRE-ChR2 and TRE-RCaMP6f for optical stimulation and imaging of HPC cells.

- The Fear Group will be conditioned such that they learn to associate a context with multiple foot shocks. Next, the group will undergo a protocol to naturally suppress the fear memory by repeatedly exposing the subjects to the context but without further administration of foot shocks—a process termed extinction. Then, in a different context, we will administer an aversive stimulus (e.g. a brief foot shock) which is sufficient to *reactivate* the extinguished fear memory—a process termed reinstatement. Experimental groups will have HPC cells that originally processed the fear memory optogenetically inhibited during the administration of the aversive stimulus in an attempt to prevent the extinguished fear memory from being reinstated. Finally, all subjects will be given a fear memory recall session a day and a month after inhibition. RCaMP6f animals will have cells imaged during extinction, reinstatement, and memory recall.
- The Positive Group will be fear conditioned and then placed back in the context for fear recall. During recall we will simultaneously stimulate HPC cells processing a

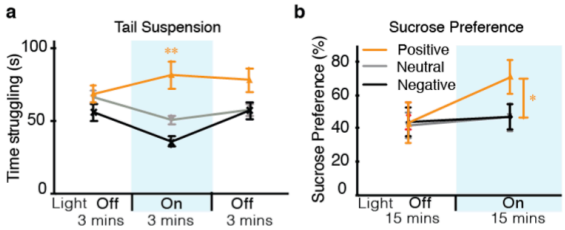

Figure 2. Activating a positive engrams rescues depression-like behavior. **(A-B)** Optical reactivation of dentate gyrus cells that were previously active during positive (orange), but not neutral (gray) or negative (black) memory formation significantly increases time struggling in the tail suspension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

positive memory to "update" the fear memory with positive information, thereby suppressing subsequent fear responses to the context. Subjects will be given a fear recall session a day and a month after positive memory activation. A separate group will also undergo an extinction protocol to measure if positive memory reactivation during fear recall expedites extinction. The RCaMP6f animals will have cells imaged during fear conditioning and recall tests a day and a month later, as well as during extinction in a separate group.

In terms of behavior, we hypothesize that the Fear Group will show a persistently suppressed fear memory during the final recall sessions a day and a month after our interventions; in terms of cellular dynamics, we hypothesize that cells that processed a fear memory will show robust ensemble activity during the initial extinction sessions as well as during reinstatement and memory recall; however, we predict that if silencing of HPC cells during reinstatement indeed abolishes fear behavior, then these ensembles will now *fail* to be active during the final memory recall tests, thus revealing the causal role of a discrete group of cells in driving aversive behavioral responses. In the Positive Group, we predict that cells processing a positive memory will be sufficient to attenuate fear memory recall as well as expedite extinction as evidenced by low levels of fear responses. Promisingly, our preliminary data suggest that positive memory reactivation during fear memory recall is sufficient to attenuate the real-time expression of fear (**Figure 3**). In the RCaMP6f Positive Group, we predict that fear and positive memories will elicit robust activity in *non-overlapping* HPC ensembles. We also predict that positive memory stimulation during fear recall will render positive memory cells more active in fear recall tests and that their activity is inversely proportional to fear behavior. Together, our experiments provide a highly novel intervention, physiological dissection, and circuit-based framework to enduringly mitigate neuronal and behavioral fear responses.

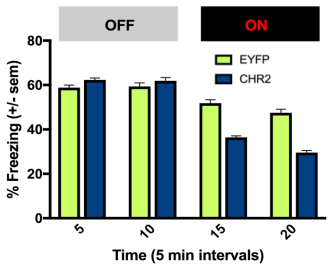

Figure 3: Activating positive memories suppresses fear expression. Subjects were fear conditioned and then given a fear recall test (above). Comparable levels of freezing are observed in the first 10 minutes (OFF) between control EYFP and experimental ChR2 groups, while in the last 10 minutes the ChR2 group shows attenuated freezing when a positive memory is reactivated (ON).

Specific Aim 2. Does chronic activation of aversive memories lastingly extinguish fear?

Rationale: Recent methods utilized to stimulate brain circuits in humans, such as deep brain stimulation, have yielded promising therapeutic responses from treatment resistant patients, thus conferring therapeutic value to chronic stimulation protocols. To gain a mechanistic understanding of how chronically activated circuits supporting memories may reprogram maladaptive states, we will test the hypothesis that repeated reactivation of HPC cells processing a negative memory is sufficient to permanently erase fear responses. The structural and functional circuitry supporting each effect will be explored using in vivo imaging to identity key cellular and physiological loci mediating the effects of chronic memory stimulation. We will utilize two groups: a Negative Memory Manipulation group and a Negative Memory Imaging group.

- The Negative Memory Manipulation group will have TRE-ChR2 expressed in cells active during fear conditioning. Next, these cells will be stimulated twice a day for 10-minute sessions across 5 days—we have recently utilized this protocol to repeatedly reactivate positive memories to successfully reverse a medley of symptoms in animal models of depression, thus validating its efficacy in lastingly reprogramming neural activity and behaviors. One day and a month later, this group will be brought back into the environment in which foot shocks were delivered to measure freezing levels. I predict that chronic stimulation of a negative memory will be sufficient to dampen the associated fear responses, thus demonstrating for the first time, to our knowledge, that repeated stimulation of negative memory HPC cells is sufficient to attenuate aversive behavioral outputs.
- The Negative Memory Imaging group will first have TRE-ChR2 expressed in HPC cells active during fear conditioning, as well as TRE-RCaMP6f expressed in two candidate areas that PTSD is thought to influence in animals and humans: the amygdala and prefrontal cortex (PFC). HPC cells will be chronically stimulated utilizing the abovementioned protocol. Before and after chronic stimu lation, amygdala and PFC cells will be imaged to measure changes in response kinetics, basal activity, and ensemble patterns when placed back in the conditioned environment. We hypothesize that a subset of amygdala and PFC cells will be active during a fearful experience and that, following HPC chronic stimulation, a *new* set of amygdala and PFC will emerge while the former set will be dampened, thus revealing a physiological marker predictive of activated or suppressed fear. The success of these experiments would address a long-sought goal of neuroscience: to discover physiological biomarkers causally related to specific maladaptive states.

Overall, with McKnight support we hope to utilize a highly innovative and intersectional approach to resolve underlying principles organizing memory formation and to demonstrate its therapeutic potential for lastingly alleviating cellular, circuit-level and behavioral abnormalities comprising psychiatric disease-like states.