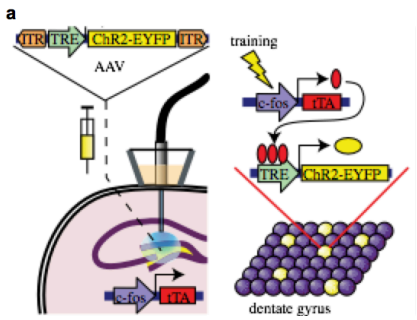

Figure 1: Engineering active hippocampus cells to express ChR2.
Mice are injected with c-fos-tTA and TRE-ChR2 into the hippocampus, followed by optic fiber implants. When Dox is not present in an animal's diet, memory formation activates the c-fos promoter which subsequently drives the expression of the transcription factor tTA. Next, tTA binds to the promoter TRE which drives the expression of ChR2, thereby labeling a population of active cells (yellow).

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 depression and 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 and novel targets to alleviate the cellular and behavioral impairments comprising depression and PTSD, which can share high co-morbidity rates and collectively affect more than 300 million people worldwide. To directly tackle this problem, we recently developed a novel genetic strategy for artificially manipulating positive, neutral and negative memories in rodent models of mood and anxiety disorders—a prospect that speaks directly with the One Mind Institute's mission to prevent and treat disorders of the brain. The significance of these proposed experiments is to artificially modulate positive and negative memories in rodents to resolve their therapeutic potential for alleviating cellular, circuit-level and behavioral abnormalities comprising depression- and PTSD-like states, as well as to discovery novel physiological signatures and behavioral interventions that enable direct translation of our findings to humans.

Aim 1: Can aversive behaviors be permanently suppressed with positive memories?

In both humans and rodents, positive experiences have been shown to increase sociability and hedonic states—which are core impairments in major depression—as well as decrease cell and behavioral stress responses. Moreover, memories are inherently modifiable and yet, the neural nodes sufficient to modulate positive and negative memories in evolutionarily conserved brain regions, such as the hippocampus (HPC), remains poorly understood. We will utilize our novel virus cocktails (Figure 1) which permit HPC cells that were active during positive memory formation to be tagged with the light sensitive ion channel ChR2 or the calcium indicator RCaMP6f for optical stimulation and *in vivo* imaging of cells. Promisingly, our recent data suggest that activated positive memories are sufficient to attenuate stress-induced impairments in motivation and hedonic-related behaviors (Figure 2).

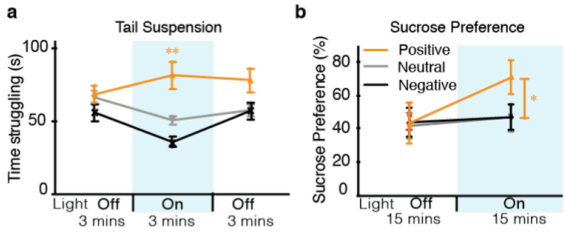

Figure 2. Activating a positive engrams rescues depression-like behavior. **(A-B)** Optical reactivation of dentate gyrus cells that were previously active during positive (orange), but not neutral (gray) or negative (black) memory formation significantly increases time struggling in the tail suspension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

Moreover, our unpublished recent data suggest that activated positive memories are sufficient to suppress real-time fear responses, though their capacity to enduringly reverse fear responses and a battery of maladaptive behaviors over time remains unknown (Figure 3). To that end, subjects will first be given a positive experience (i.e. exposing male mice to female mice) to tag active HPC cells. Next, subjects will be given an aversive experience (i.e. fear conditioned) and the following day will be placed back into the aversive environment for a fear recall session, during which we will simultaneously stimulate HPC-mediated positive memories in an attempt to "update" the fear memory with positive information, thereby suppressing subsequent fear responses. All subjects will then be given extinction, reinstatement, and spontaneous recovery sessions to measure if positive memory reactivation permanently attenuates aversive behavior. A separate group of animals will undergo the same protocol but will be given social interaction and sucrose preference assays to measure if positive memory reactivation during fear recall is sufficient to prevent long-term impairments known to be induced by stress on social and hedonic-related behaviors.

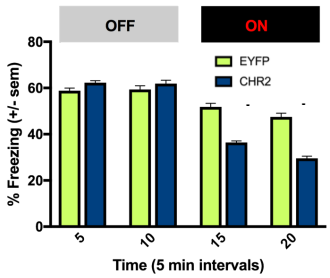

Figure 3: Activated positive memories suppress fear. Subjects were fear conditioned and then given a fear recall test. Comparable levels of freezing are observed in the first 10 minutes (OFF) between control EYFP and experimental ChR2 groups; when a positive memory is reactivated (ON) in the last 10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

Next, to visualize ensemble dynamics in real-time and extract neural codes specific to memory updating with positive information, separate RCaMP6f groups—in which previously active cells were tagged for imaging—will undergo the same protocols for imaging in two candidate areas known to be affected in patients with depression and PTSD: the amygdala and prefrontal cortex (PFC). We predict that activated positive memories will be sufficient to acutely and permanently attenuate fear as well

as prevent fear-induced decreases in social and hedonic-like behaviors. In the RCaMP6f group, we predict that amygdala and PFC activity of positive memory populations will be inversely proportional to fear behavior and directly proportional to increases in hedonic and social behaviors. Collectively, these data would thereby provide a physiological link between neural activity and positive or negative memory recall, as well as physiological readouts of social and hedonic-like states, while simultaneously providing such predictive physiological signatures for subsequent human imaging research in patients with depression and/or PTSD.

Aim 2. Does chronic activation of negative memories permanently alter aversive behaviors?

Recent methods to stimulate brain circuits in humans, (e.g. deep brain stimulation), have yielded lasting behavioral changes in patients with treatment-resistant depression; yet, the neuronal underpinnings sufficient to support such interventions remain elusive. To gain a mechanistic understanding of how chronically activated circuits supporting aversive memories may reprogram behavioral states, we will test the hypothesis that repeated reactivation of HPC cells processing a negative memory is sufficient to permanently suppress 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 negative memory stimulation in the aforementioned areas known to be crucial for emotional outputs in humans and rodents: the amygdala and PFC. We will utilize 2 groups: a Negative Memory Manipulation group and a Negative Memory Imaging group.

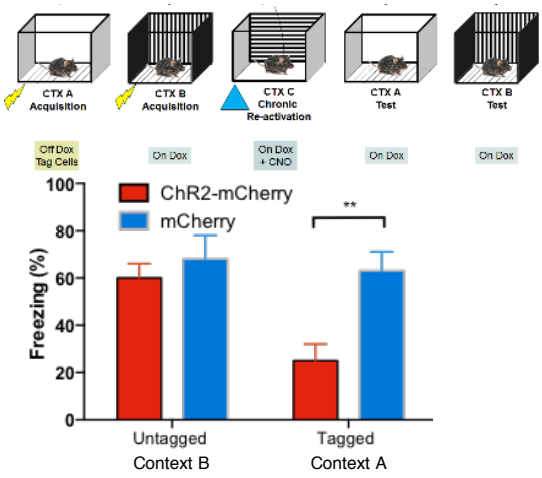

Figure 4: Subjects were first fear conditioned in context A and active HPC cells were tagged with ChR2. Next, these subjects were fear conditioned in context B but without further tagging of any HPC cells. In context C, cells that were originally active and tagged in context A were chronically stimulated. When place back in context A, the experimental ChR2-mCherry group displayed robust decreases in freezing relative to mCherry controls; moreover, the effect was context-specific and did not elicit any changes in fear behavior when placed back in untagged context B.

Utilizing a similar genetic tagging strategy from Aim 1, the Negative Memory Manipulation group will have ChR2 expressed in cells that are specifically 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. First, this group will undergo chronic stimulation of HPC cells processing a negative memory. All subjects will then be given extinction, reinstatement, and spontaneous recovery sessions to measure if chronic negative memory reactivation permanently attenuates aversive behavior. A separate group of subjects will also undergo the same chronic stimulation procedure, followed by social interaction and sucrose preference assays to measure if chronic negative memory stimulation is sufficient to precipitate social and anhedonic-like behavioral impairments, thereby providing insight into the neural mechanisms underlying two core depression-related states. As a proof of principle, our recent data suggest that chronically activating HPC cells processing a negative memory is sufficient to suppress fear responses in a context-specific manner 24 hours after stimulation (Figure 4).

The Negative Memory Imaging group will first have ChR2 expressed in HPC cells that were previously active during fear conditioning, as well as RCaMP6f expressed the amygdala and PFC (as before, and to prevent cross-talk between optogenetic stimulation and RCaMP6f visualization). HPC cells will first be chronically stimulated utilizing the abovementioned protocol. Before and after chronic stimulation, 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, as well as during sucrose and social interaction assays. We hypothesize that a subset of amygdala and PFC cells will be active during a fearful experience and that, following chronic HPC stimulation, an anatomically and physiologically distinct set of amygdala and PFC will emerge while the former set will be suppressed, thus revealing a physiological marker predictive of activated or suppressed aversive states. The success of these experiments would address a long-sought goal of neuroscience, namely, to discover physiological biomarkers causally related to specific maladaptive states. Overall, with the One Mind Institute's 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 in general and stress-induced maladaptive states in particular.