The Dissertation Committee for Emma Taylor Brockway Certifies that this is the approved version of the following Dissertation:

Ventral Hippocampal Regulation of Contextual Fear and Extinction Memory

Committee:

Michael R. Drew, Supervisor

Boris V. Zemelman

Laura Colgin

Joseph Dunsmoor

Stephen Maren

Ventral Hippocampal Regulation of Contextual Fear and Extinction Memory

by

Emma Taylor Brockway

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

December 2023

Dedication

To my cats, Tigerlily and Obsidian, whose learning and behavior never quite fit my expected models, but whose behavior has gotten me through these experiments and writing, nonetheless.

Abstract

Ventral Hippocampal Regulation of Contextual Fear and Extinction Memory

Emma Taylor Brockway, PhD

The University of Texas at Austin, 2023

Supervisor: Michael R. Drew

Fear learning creates long-term memories through which predictive cues or the context surrounding the fearful event acquire negative associations. Later exposure to these stimuli elicits a fearful response, but this learned response will diminish in the absence of threat, a process known as extinction. Extinction does not abolish fear memory, but instead creates a separate memory of safety. The hippocampus is thought to be a hub for competition of the expression of these two opposing memories. Neural ensemble representations of contextual fear and extinction memories are distinct in the hippocampus, but how these memories are processed to influence recall and behavior is not known. These experiments sought to investigate activity in the ventral CA1 and subiculum (vHP), where projections to other fear and extinction related structures are located, to better understand how the hippocampus influences the expression and suppression of fear behavior. First, we investigated whether activity among vHP projections to the BLA and IL differed during context fear and extinction recall. We found that fear recall causes more activation of projections to BLA compared to IL, while extinction recall results in the opposite pattern

of more activation of projections to IL than BLA. This shows that the ventral hippocampus is sensitive to the valence of contextual memory, and signals to relevant brain regions based on that valence. Next, we sought to selectively inhibit the projections from vHP to BLA and IL to test if these projections are indeed necessary for further recall of these memories. These manipulations were unsuccessful in impairing recall. Finally, we stimulated SST interneurons in vHP or IL to induce feed-forward inhibition. We found that stimulating vHP SST interneurons impaired fear recall, reducing fear behavior, and impaired extinction learning, resulting in higher fear behavior in a later test. This result demonstrates the vHP's role in both context fear expression and suppression. Increasing inhibition in the IL did not affect context fear or extinction recall but did impair auditory cue extinction. Overall, these results provide evidence that vHP activity modulates context memory in a valence dependent way through connections to other fear related regions.

Table of Contents

List of Figures

Chapter 1: Introduction

Memory of fear and safety are opposing in nature, yet often intricately tied together. As one learns to overcome a particular fear, the memory of it does not go away, but new memories of safety are made that can guide behavior. The hippocampus, a region long studied for its role in memory, is thought to be a crucial region for representation and competition of these memories. Yet how the hippocampus processes these opposing memories and signals to other brain regions to initiate corresponding behavioral responses is not fully understood.

Memory consists of enduring plasticity across a widely distributed population of neurons in the brain (Josselyn et al., 2015). Work by Karl Lashley to identify the location of memories in the brain demonstrated that lesions of many different cortical areas could cause memory impairment, and led to the idea that memory is distributed across brain regions (Lashley, 1950). However, some regions, like the hippocampus, have an outsized role in forming and recalling particular types of memory. This was famously demonstrated in the case of Henry Molaison (patient H.M.), in which both lobes of H.M.'s temporal lobes, containing most of his hippocampus, were removed to treat his severe epilepsy (Scoville and Milner, 1957). After the surgery, H.M. could no longer form new declarative memories (anterograde amnesia) and could only recall events that happened long in the past (temporally-graded retrograde amnesia). As H.M. could still perform other memory tasks like motor-learning and short-term memory, this finding advanced the idea that specific brain regions preferentially mediate certain types of memory and that the hippocampus was necessary for formation of long-term declarative memories.

Fearful experiences create long lasting memories, in which cues and the context surrounding the fearful event gain negative associations (Gale et al., 2004). Exposure to

fear-associated cues or contexts at a later time elicits a fearful response, but repeated presentations of these stimuli in the absence of threat will cause learned fear to gradually diminish, a process known as extinction. However, extinction is not unlearning of fear, but rather a new learning experience of safety, and fear often relapses. Investigating how distinct memories of fear and extinction are expressed to activate or suppress fear behavior can lead to better therapies as well as increase our understanding of memory processing in the brain.

Recent studies have isolated memory traces to particular ensembles of neurons (Josselyn, 2010; Josselyn et al., 2015). The plasticity changes during learning in these particular neurons and their later reactivation gives rise to recall of memory (Liu et al., 2012a; Park et al., 2016). Neurons active in the hippocampus during learning of fear memory can be selectively labeled, and activating or inhibiting this ensemble can increase or decrease fear respectively (Liu et al., 2012a; Chen et al., 2019). After extinction of fear, when fear responses are low, a memory of relative safety can be similarly targeted and manipulated (Lacagnina et al., 2019). However, in this case increasing activity of the ensemble leads to decreased fear behavior, and inhibiting the extinction ensemble leads to increased fear behavior. While the hippocampus maintains memory representations of both fear and extinction memories, how these otherwise similar sets of neurons give rise to opposing behavioral responses based on the associated valence of the memory is not well understood.

As more has been learned about hippocampal memory ensembles, there has been increasing focus to understand how the hippocampus functions within larger circuits to initiate proper recall of memory and lead to memory-appropriate behavioral responses. This dissertation centers on that question: how does the hippocampus output information to evoke behavior that is appropriate to a memory? To investigate this question, this

dissertation will provide an overview of fear and extinction learning and memory and what is known about the hippocampus and larger circuitry involved, and then describe experiments performed to investigate the role of the ventral hippocampus in fear and extinction memory processing.

1.1 Learning of Fear and Safety

1.1.1 Fear Conditioning

Pavlovian fear conditioning is a widely studied model of associative learning. At its roots, Pavlovian conditioning describes a learned association between stimuli and outcomes (Rescorla, 1987). An aversive stimulus (unconditioned stimulus, US) naturally can cause a behavioral response (unconditioned response, UR). In the classic example, Pavlov's dogs would salivate (UR) when presented with food (US). By pairing a neutral stimulus (conditioned stimulus, CS) such as ringing a bell right before the US, an association between the two is learned, such that the new stimulus also produces a conditioned response (CR), and the dogs then salivate when they hear the bell (Rescorla, 1987). Pavlovian conditioning allows the subject to learn about associations and consequences of the things they experience and use that memory to predict and interact with their environment.

In fear conditioning, by pairing a neutral stimulus with an aversive stimulus, the neutral stimulus acquires the ability to elicit defensive or fearful responses. Fear memory is an adaptive form of learning and is incredibly conserved amongst species (Pape and Pare, 2010). An animal that learns to associate predictive cues with an approaching predator is more likely to live on. The behaviors we might consider "fear" behavior change species to species, but associative conditioning can widely be used to describe this type of learning

(Bolles, 1970; Crawford and Masterson, 1982). Cued fear conditioning paradigms are commonly used in rodents where a discrete stimulus such as an auditory tone or light are presented for a few second then culminate in a short foot shock delivered through an electrified grid flooring (Rescorla, 1968, 1987). Upon later presentation of the cue, subjects will display freezing behavior, or the absence of all movement other than breathing, which is an adaptive fear response in rodents to avoid detection by predators (Bouton and Bolles, 1980). Context fear conditioning is a form of conditioning where there is no discrete cue as the conditioned stimulus, but instead the assortment of features that make up the environmental background are associated with the aversive stimulus (eg. a foot shock) (Fanselow, 1990, 2000). As a result, subjects will display freezing when returned to the context.

1.1.2 Fear Extinction

Fear extinction describes an opposing form of learning to fear conditioning in which fear responses decrease over repeated exposure to a CS that is not followed by an aversive stimulus (Bouton and Bolles, 1979). Fear memory can serve an adaptive function to help organisms recognize dangerous situations and apply past knowledge to avoid painful outcomes. However, it is equally important to learn when those associations are no longer applicable and adapt behavior accordingly. This is often modeled through repeated presentations of a discrete CS in one session, or repeated re-exposure to the conditioned context over multiple sessions. Fear responses will gradually attenuate across the presentations.

Extinction learning is not considered unlearning of the original fear association, but rather the new learning of a competing association of safety that may inhibit fear memory and responses (Lattal et al., 2006). This can be seen through mechanisms such as

spontaneous recovery of fear, where fear responses return over time, demonstrating that the fear memory is still present and can be recalled (Shurtleff and Ayres, 1981). Fear responses can also be renewed by presenting the extinguished CS in a context other than the extinction context, or reinstated by presenting the US non-contingent with the CS (Bouton and Bolles, 1979; Bouton and King, 1983). Renewal demonstrates that extinction is highly context dependent. Bouton and King (1983) compared groups of rats that were fear conditioned to an auditory tone in one context (A) then received extinction trials in either the original fear context (A) or an alternate context (B). Although both groups show similar loss of conditioned fear during extinction, when both groups were tested in context A, the rats that received extinction in context A showed less fear, whereas the rats extinguished in an alternate context (B) had higher fear. This shows that the expression of extinction is highly sensitive to contextual cues. Bouton suggested that contextual cues serve to disambiguate the significance of a stimulus, helping to identify when a situation is predictive of shock and allowing for adaptive behavior as conditions change (Bouton, 1993).

1.1.3 Neural Circuits of Fear and Extinction

The neural circuitry underlying fear learning and memory consists of a distributed network of regions working in concert to control acquisition, consolidation, and expression. Each structure contributes differently to these learning and memory processes, and the interconnectivity of these regions gives rise to complex and finely controlled memory recall and behavior.

The amygdala, particularly the basolateral complex of the amygdala (BLA), is essential for fear conditioning (Fanselow and Ledoux, 1999). The amygdala receives input from cortical and thalamic areas containing sensory information about the CS and US, and

the spatiotemporal association of this input causes Hebbian plasticity (Ledoux, 2000; Pape and Pare, 2010). Information is then routed to the central amygdala, which projects to hypothalamic and brainstem targets to initiate defensive behavior. Lesions of the amygdala prior to fear learning block the acquisition of conditioned fear responses, and lesions a month after fear learning can block the recall and expression of fear (Davis, 1992; Maren et al., 1996a). The firing and plasticity of amygdala neurons is also critical for learning and expression of conditioned fear. Fear conditioning enhances the responses of amygdala neurons, and acquisition of fear conditioning can also be blocked with an N-methyl-D-aspartate (NMDA) receptor antagonist (Quirk et al., 1995; Maren et al., 1996b; Maren and Quirk, 2004). Human studies have also shown that amygdala activation corresponds to fear expression and that amygdalar ablation leads to reduced and abnormal fear expression (Vytal and Hamann, 2010; Feinstein et al., 2011). The large number of studies on the amygdala have positioned this region as the main hub for fear learning and expression.

The amygdala is also integral to fear extinction. Acquisition of fear extinction is impaired by blocking NR2B-containing NMDA receptors or mitogen-activated protein kinase/extracellular-signal regulated kinase (MAPK/ERK) in the BLA (Herry et al., 2006; Sotres-Bayon et al., 2007). Distinct groups of neurons in the amygdala appear to encode fear and extinction, with the fear population showing an excitatory response to the fear cue after fear conditioning and the extinction population initially showing no response to the cue but increasing responding late in extinction (Herry et al., 2008; Grewe et al., 2017). The intercalated cells, a GABAergic population between the basolateral amygdala and the central amygdala, may mediate the response of these extinction-activated neurons, as the intercalated cells are required for expression of fear extinction and show increased inhibition onto central amygdala neurons after extinction (Likhtik et al., 2008; Amano et al., 2010). Altogether, the amygdala is essential for both fear and extinction.

The medial prefrontal cortex (mPFC) asserts top-down control of the amygdala, and greatly influences fear expression and suppression. In rodents, the mPFC is subdivided into infralimbic (IL) and prelimbic (PL) regions. Early studies implicated the mPFC in behavioral control of fear, but not learning of fear and extinction. Lesions of the entire mPFC did not have an effect on the level of fear expression to context or cued fear conditioning, but these animals took longer to reach extinction criterion (Morgan et al., 1993). In a follow up study, selective lesions of the PL demonstrated a general increase in fear during both acquisition and extinction, suggesting the PL mediated the amount of conditioned fear expressed (Morgan and LeDoux, 1995). Electrolytic lesions of the vmPFC (including IL and some PL) before fear conditioning had no effect on fear conditioning or extinction training, but were apparent the following day when lesioned animals displayed significantly higher fear (Quirk et al., 2000). This was taken to mean that IL was not necessary for learning extinction within training but was necessary for the consolidation and later retrieval of extinction memory.

Further characterization of the PL and IL have confirmed their opposing influences on fear expressions and suppression, while also showing that these regions are involved in fear and extinction learning to some extent. Recordings show increased PL responding during the presentation of the CS which correlates with freezing behavior across both acquisition and extinction phases (Milad and Quirk, 2002; Burgos-Robles et al., 2009). The persistence of PL activity also correlates with failure to express extinction, or persistence of fear behavior after extinction. Inactivation of the PL during extinction causes reduced freezing within the session, but has no effect on learning extinction as shown in later recall tests (Laurent and Westbrook, 2009; Sierra-Mercado et al., 2011). These studies demonstrate that PL activity influences the expression of conditioned fear, but there is some evidence that PL is involved in acquisition of learned fear. In a contextual

discrimination task, where there are two CS's and one is paired with shock only in context A and the other CS is only paired with shock in context B, PL inactivation interferes with encoding and expression of appropriate CS responding (Sharpe and Killcross, 2015). Additionally, inactivation of PL following brief fear memory retrieval has been shown to block reconsolidation of the memory, resulting in impaired fear memory and reinstatement at later times (Stern et al., 2014). Altogether, the PL is a key influence on the expression of fear and may be involved in acquisition and maintenance of learned fear depending on specific task parameters.

Further investigation of the IL has similarly confirmed its role in the suppression of fear while elucidating its role in extinction learning and consolidation. Inactivating IL during extinction recall impairs expression of learned extinction (Laurent and Westbrook, 2009). Recordings of IL neurons show increased activity during extinction recall, and electrically stimulating the IL when the CS is presented during extinction leads to better extinction recall later (less fear behavior) (Milad and Quirk, 2002). Pharmacological inactivation of IL during extinction learning also impairs extinction recall (Sierra-Mercado et al., 2011). These results show that the IL is involved in the suppression of fear during extinction learning and retrieval. Additionally, simultaneous recordings of both IL and PL have demonstrated that the relative balance of activity between the two regions correlates with the amount of fear behavior that is displayed, where higher PL activity was seen along with higher levels of fear (Giustino et al., 2016). The PL and IL appear to work in tandem to influence the expression or suppression of fear, respectively. The studies showing impaired learning and memory consolidation further suggest that the mPFC plays a role in learning and maintenance of memory. The contrasting influences of the mPFC suggest that it plays a role in higher-order memory, which may be especially important in extinction,

when new associations are learned, and in cases like the discrimination task, where judgements based on competing associations guide behavior.

mPFC is believed to influence extinction learning through projections to the BLA. In naïve mice, IL and PL projections recruit equivalent excitation and feedforward inhibition of principal neurons in the BLA. However, fear conditioning causes a selective decrease in the inhibition:excitation balance in the PL circuit only, suggesting the PL to BLA pathway undergoes synaptic plasticity during fear learning (Arruda-Carvalho and Clem, 2014). On the other hand, optogenetically stimulating or inhibiting the IL to BLA projections during extinction acquisition results in better extinction recall and worse extinction recall respectively (Bukalo et al., 2015). Recordings of IL and BLA also demonstrate that theta coupling (synchronous activity between these regions) is high early in extinction learning and when discriminating a safe cue from an aversive cue (Likhtik et al., 2014). Based on these and other studies, projections from PFC to BLA are believed to influence extinction learning but not the expression of extinction.

Finally, the hippocampus is traditionally thought to process contextual cues related to when and where to express or suppress learned fear. Lesioning the hippocampus shortly after auditory fear conditioning greatly reduces freezing to the fear context but not to a learned tone (Kim and Fanselow, 1992; Maren et al., 1997). Rats that received lesions one day after fear conditioning display little to no freezing to the fear context, however rats that were lesioned at longer intervals after conditioning retained significant context memory (Kim and Fanselow, 1992). Rats in all lesion groups displayed similar freezing to the conditioned tone. Because of this temporal degradation of context memory but not a discrete tone, the hippocampus is thought to facilitate the rapid learning of conjunctive representations of environmental features. However, the hippocampus is not needed for recall of remote context memories, and rats without a hippocampus can still learn context

conditioning when a task is extensively trained, showing some compensation and recruitment of other brain regions for context memory (Wiltgen, 2006). In these and many other studies, the hippocampus has been shown to play an integral role in contextual memory.

Additionally, extinction is highly context-dependent and the context becomes a powerful predictor of whether an auditory cue will signal shock (Bouton et al., 2006). When recall is tested in the environment in which extinction was learned, extinction is well recalled and fear is low: when extinction tested in the original fear context or a new context, fear is renewed (Bouton and King, 1983). Pharmacologically inactivating the hippocampus attenuates this renewal of fear to an extinguished CS when it is presented outside of the extinction context and disrupts context-specific firing patterns of amygdala neurons to the extinguished CS, demonstrating that the hippocampus processes the contextual cues involved in fear conditioning (Corcoran and Maren, 2001; Maren and Hobin, 2007). Fear renewal can also be blocked by inhibiting the input from the ventral hippocampus to the IL (Marek et al., 2018). Thus, the hippocampus exerts contextual control over cued fear expression and suppression through its connections with the amygdala and PFC.

The hippocampus generates a conjunctive representation of context, binding together multi-sensory features of the environment (Fanselow, 2000; O'Reilly and Rudy, 2001). Traditionally, after context-shock pairings this context representation acquires the ability to elicit fear through projections to the amygdala (Ledoux, 2000; Maren and Quirk, 2004). This view positions the hippocampal context representation as a static CS, where conditioning and extinction do not alter the representation itself but do alter the ability of the context representation to elicit fear. Many studies have indeed shown that context memory formation is a separate and necessary process in order for context fear conditioning to occur. When mice are not given adequate time to process the context, and

a shock is delivered immediately after being placed in the context, mice will not display learned fear to the context in later tests (Fanselow, 1990). However, if mice are given time to explore and learn the context a day prior the same immediately-delivered shock will result in conditioned fear to the context, suggesting that an existing context representation is being recalled and enabling the learning of a context-US association (Rudy et al., 2002). The hippocampal role in context fear conditioning and cued fear conditioning is mediated through projections to the amygdala. Optogenetic inhibition of ventral hippocampal projections to the basal amygdala reduces freezing to a conditioned context, whereas inhibiting a parallel projection to the central amygdala impairs context-dependent renewal of cued fear memory (Xu et al., 2016). These studies show that the hippocampus contributes critical information about context during conditioning.

However, recent studies have demonstrated that hippocampal context representations are not static, and hippocampal plasticity and activity contribute to distinguishing memory for fear and safety within the same context. Memory representations are thought to consist of enduring plasticity in ensembles of neurons. Hippocampal CA1 place cells receive sensory input and are highly selective, firing action potentials in one preferred location in space. Place cells are typically stable in a context, representing a stable memory trace related to the environment, and remapping (meaning the place fields move location, appear, or disappear) is usually associated with a change to a different context altogether (Colgin et al., 2008). However, CA1 place cells have been demonstrated to remap during fear conditioning, suggesting that the context changed enough to require a new representation (Moita, 2004). Indeed, the new place maps remained stable for a period of days (Wang et al., 2012). Remapping also occurs during fear extinction, suggesting that the contextual representation of space alters during fear and

extinction learning to encode emotional valence in addition to spatial information (Wang et al., 2015).

Studies using immediate-early gene tagging systems have further identified distinct ensembles of hippocampal neurons in the dentate gyrus that represent fear and extinction memory. These strategies take advantage of the fact that immediate early genes (IEGs), such as c-Fos and Arc, become expressed in response to neural activity (Greenburg and Ziff, 1984; Morgan and Curran, 1989). Transgenic mice have been engineered to so that expression of an IEG additionally drives the transcription of a reporter to label and target the cell. The window of tagging of these systems is limited by requiring another component for recombination, such as the introduction of 4-hydroxytomoxifen for the ArcCreERT^2 system (Denny et al., 2014). These mice allow for the tagging of neurons active during a particular experience and then comparison to IEG's active in neurons during a later experience. When neural ensembles are tagged during fear conditioning and compared to neural activation following context fear retrieval, a higher degree of reactivated neurons are seen in the DG and CA3 than in a mouse exposed to a novel context (Denny et al., 2014). Reactivation of the original tagged ensemble is believed to underly recall of the memory. Reactivation is both necessary and sufficient for recall, as optogenetically silencing this fear-tagged ensemble was demonstrated to impair fear expression, whereas stimulating a fear-tagged ensemble has been shown to induce freezing in a novel context (Liu et al., 2012b; Denny et al., 2014).

Work in our lab by Anthony Lacagnina further investigated the influence of extinction on a fear-tagged ensemble. He found that mice that received extinction exhibited less reactivation of the fear-tagged ensemble than mice who did not receive extinction and were tested for recall of fear (Lacagnina et al., 2019). In a second experiment, an extinction ensemble was tagged after 10 days of extinction, and this ensemble showed high

reactivation when tested 5 days later when fear was low. In contrast, fear-tagged neurons were again highly reactivated 28 days after extinction, when animals showed spontaneous recovery of fear. These results demonstrated that fear and extinction were distinctly represented in hippocampal neural ensembles, and that reactivation of these neurons corresponded to the level of activation of fear. Further, Lacagnina et al. demonstrated that optogenetic silencing of the extinction-tagged ensemble increased fear after extinction, whereas silencing the fear-tagged ensemble reduced fear during spontaneous recovery. Altogether, these studies show that fear and extinction are encoded in distinct ensembles in the hippocampus that can influence fear behavior in opposing manners. This suggests that hippocampal context representations change during extinction to encode the valence of the memory and influence behavior accordingly, rather than maintaining a stable representation of context that acquires fear association in the amygdala like previously thought.

1.2 Memory and Function in The Hippocampus

The hippocampus is a hub for the construction of multisensory features into a representation of a spatial environment in a way that can support complex memory of that space and influence behavior accordingly. Further investigation into the structure and other functions of the hippocampus may lead to a better understanding of how the hippocampus processes memory and signals this information to affect behavior. The hippocampus is made up of multiple subregions, namely the dentate gyrus (DG), CA3, and CA1. Hippocampal memory functions are thought to occur through processes of pattern separation and completion (Rolls, 1996, 2018; Leutgeb et al., 2007; Kesner and Rolls, 2015). This computational theory of hippocampal function has been extensively described

along with tests of this theory by Kesner and Rolls (2015) and is briefly summarized in the rest of this paragraph. Cortical sensory input from the entorhinal cortex first goes to the DG, where a process of pattern separation occurs. Interneuron activity in the DG keeps cells firing at a low rate, allowing for sparse activation of neurons during memory encoding that allows for distinct patterns of ensemble activity. Orthogonalized patterns of sparse activity in the DG are thought to allow for separation of memories for rapid learning without damaging previously existing representations. DG sends information through the mossy fibers to the CA3, where pattern completion occurs. CA3 is considered a single-attractor or auto-association network that allows for rapid one-trial associations between features as well as the ability to complete a whole memory from the recall of one part. While DG pattern separation is critical to learning distinct memories, during recall sensory information can directly project from the entorhinal cortex to CA3 through the perforant path, where CA3 recurrent collaterals allow for recall of a full featured representation. This information is then processed in the CA1, which sends projections to cortical areas to reinstate the memory representation in other cortical areas and project to subcortical areas to guide actions. These theories of pattern separation and pattern completion are thought to allow the hippocampus to rapidly acquire and store distinct memories, and to reactivate full memory representations based on partial features.

The hippocampus is further subdivided into dorsal (or anterior) and ventral (posterior) segments that have different input and output connectivity to other brain regions. The aforementioned studies identifying distinct fear and extinction ensemble representations during recall have focused on the dorsal DG and CA3 (Liu et al., 2012b; Denny et al., 2014; Lacagnina et al., 2019), but the projections to the prefrontal cortex and amygdala that may influence fear behavior originate from the ventral CA1 and subiculum. There is still much to understand about how fear memory may be differently represented

and conveyed through the trisynaptic circuit and to the ventral output regions, and how ventral hippocampal projections influence other brain regions involved in fear memory expression. Here I will discuss some of the research on other types of memory and behavior in the hippocampus, namely spatial processing and behavioral regulation, to help put fear context memory into the context of broader hippocampal function.

1.2.1 Functional Differentiation of Dorsal and Ventral Hippocampus

The hippocampus is a collection of distinct subfields classified by their cell types and projection patterns. Along the transverse axis of the hippocampus is the trisynaptic circuit, where synaptic transmission flows from the granule cells of the dentate gyrus via mossy fibers to the pyramidal cells of the CA3, then via Shaffer collaterals to the pyramidal cells of the CA1. If the hippocampus were a swiss roll, taking a cross section slice of the roll would reveal the cellular layers and subregions like the icing going through, with the same swirl pattern consistently represented through the long cake no matter where you slice it. The transverse connections (the slice) in this elongated brain region are repeated throughout the longitudinal or septotemporal axis (the length of the swiss roll) in such a way that it was once thought to have independent laminar structure (Andersen et al., 1971), but the hippocampus has indeed been shown to have internal connections along the septotemporal axis. Associational projections in the DG and CA3, neurons that contact back onto dendrites within their cellular layer, span long lengths of the septotemporal axis and consist of two largely non-overlapping clusters, one of which spans approximately the dorsal two thirds and the other the remaining ventral one third (Swanson et al., 1978). Importantly, these associational projections differentiate the dorsal and ventral poles of this axis, which have quite different external connectivity (Amaral and Witter, 1989). While the CA1 does not have associational projections, CA1 pyramidal neurons project broadly

to the subiculum, with individual axonal branches extending to as much as one third of the transverse axis of the subiculum (Tamamaki et al., 1987; Witter and Amaral, 1991). Interestingly, the subiculum gives rise to a largely unidirectional longitudinal associational projection that extends from the level of the cells of origin to much of the subiculum located ventrally (Harris et al., 2001). Associational projections among the cell layers show both how the hippocampus is distinctly segmented along the septotemporal axis, and how communication along this axis can occur.

A difference in afferent sensory cortical connections provides these two hippocampal regions with access to different amounts and types of sensory information. The wide-ranging sensory input that is conveyed to the hippocampus is routed through the entorhinal cortex (EC). Within the EC there are three band-like zones that span across the defined subfields of the region. These bands are only sparsely interconnected and project to specific areas of the hippocampal dentate gyrus. The caudolateral zone of the EC innervates the septal (dorsal) half of the DG, the intermediate zone the adjacent quarter, and the rostomedial zone the most temporal (ventral) quarter (Witter, 1986; Dolorfo and Amaral, 1998). Because of this division, the various areas of the hippocampus receive separate sensory cortical information from the perirhinal and postrhinal cortices via the EC (For review, see: Witter, 1986; Witter et al., 2017). The caudolateral EC band receives the most visuospatial and other sensory information from the perirhinal cortex, which is conveyed to the dorsal two thirds of the hippocampus, and the medial band, which projects temporally, primarily receives hypothalamic and olfactory area inputs. The intermediate band of the EC receives widespread sensory input. While there is some overlap in conveyed sensory information, namely coming from the olfactory cortex, there is a significant division in these pathways along the dorsal to ventral axis, meaning that spatial sensory information is predominantly relayed to the dorsal region. So the dorsal and intermediate

regions receive visuospatial and other widespread sensory input and have associational connections between them, while the ventral region is distinctly separate, and receives the bulk of olfactory, gustatory, and visceral inputs.

External hippocampal projections are also topographical. Axons from CA1 and the subiculum, two main output areas of the hippocampus, terminate in areas of EC that they receive information from, where dorsal projections terminate more laterally in the EC and ventral projections terminate in the medial area (Köhler, 1985; van Groen et al., 1986). Efferent hippocampal projections also differentially innervate subcortical structures topographically. Hippocampal projections to the lateral septum (LS) are graded in that the dorsal hippocampus projects to the dorsal LS and progressively more ventral regions in the hippocampus project to progressively larger and more ventral regions of the LS (Swanson and Cowan, 1977; Risold and Swanson, 1996). The lateral septum in turn projects topographically to various hypothalamic areas, providing various means of affecting behavior.

Analysis of other dorsal and ventral efferent projections further suggests a functional separation based on the roles of structures the dorsal and ventral hippocampus project to. The dorsal CA1 and subiculum have two prominent projections to the retrosplenial and anterior cingulate cortices (Cenquizca and Swanson, 2007), regions that are involved in processing visuospatial information and spatial navigation (Frankland et al., 2004; Troy Harker and Whishaw, 2004). Along with projections to the mammillary nuclei and anterior thalamus, which contain navigation-related head direction neurons (Taube, 2007), these pathways provide a mechanism for the hippocampus's influence on spatial memory. Additionally, the dorsal hippocampus can influence the ventral tegmental area (VTA) and substantia nigra (SNr) by way of projections to the rostrolateral nucleus accumbens and rostral caudoputamen. The VTA and SNr are important for locomotion and

orienting movements of the eye, head and neck, as well as dopamine projections that are crucial for reward and motivation (Swanson and Kalivas, 2000; Gruber and McDonald, 2012). Overall, the connection of the dorsal hippocampus to these regions suggests a network capable of processing spatial information and coordinating locomotion and navigation.

While the dorsal region appears poised to process the bulk of sensory spatial information, the ventral hippocampus has afferent and efferent connections with limbic and hypothalamic regions involved in arousal and emotional regulation. The ventral CA1 region sends projections to the olfactory bulb and associated cortical areas, regions which have been implicated in depression-like symptoms (Cenquizca and Swanson, 2007; Wang et al., 2007; Fanselow and Dong, 2010). The ventral hippocampus also sends projections to the amygdala, lateral septum, medial prefrontal cortex, and bed nucleus of the stria terminalis (BNST), providing a network of influences on downstream hypothalamic nuclei that regulate neuroendocrine, autonomic, and somatic motor activities involved in motivated behaviors like sexual reproduction, maternal behavior, social behavior, feeding and defense. Projections to the amygdala, an essential structure for Pavlovian fear conditioning, arise most substantially from ventral CA1 and subiculum, and the amygdala reciprocates projections to the ventral hippocampus (Van Groen and Wyss, 1990; Petrovich et al., 2001; Kishi et al., 2006; Cenquizca and Swanson, 2007). The ventral CA1 and subiculum also send projections to the caudomedial nucleus accumbens (shell), which is integral to reward processing and motivated behavior (Gruber et al., 2009; Wassum et al., 2009). The ventral hippocampus also has a higher density of dopaminergic, noradrenergic, and serotonergic terminals than the dorsal hippocampus (Gage and Thompson, 1980; Verney et al., 1985). Altogether, the connectivity of the ventral region of the hippocampus suggests that it receives input relevant to and modulates the activity of neuroendocrine,

motivational, and emotional systems, in contrast to the spatial- and navigation-related function of the dorsal hippocampus.

1.2.2 Spatial Memory

The hippocampus has been extensively studied for its involvement in spatial memory. Hippocampal neurons in CA1 are well known for their role as place cells, as they activate preferentially in a particular location in space (O'Keefe and Dostrovsky, 1971). These cells have low baseline firing rates, with marked increase in firing when a rat orients towards and moves through a particular place, deemed a place field. O'Keefe and Nadel theorized that the hippocampus serves as a spatial reference map, providing other brain regions with information about where the animal is in the environment (O'Keefe and Nadel, 1978). They further posited that the ability of the hippocampus to represent space may allow it to serve as a cognitive map like that proposed by Tolman, a mental representation of space and context that can support memory storage and be used for prediction and guidance (Tolman, 1948; O'Keefe and Nadel, 1978). Since then, spatial memory tasks such as the Morris water maze and T-maze have been used to show that hippocampal damage causes dramatic impairment in spatial processing and memory (Morris et al., 1982). In the Morris water maze, rats with total hippocampal lesions, superficial cortical lesions, or sham-operated animals are tasked with learning the location of a platform hidden in a pool of opaque water in order to escape swimming in the pool. Although control groups learn this task rapidly, hippocampal lesioned rats have significant impairment, taking much longer, circuitous routes to find the platform (Morris et al., 1982; Moser et al., 1993).

Lesion studies show that dorsal hippocampus plays a larger role in spatial learning than ventral hippocampus. Work by the Mosers showed that lesioning as little as 20% of

the dorsal end caused longer latencies for mice to find a hidden platform in the task, with progressively larger lesions leading to linear increases in latency, whereas behavioral impairment on this task was only seen after lesions of at least 40% of the ventral hippocampus, and in these cases the escape latencies were still lower than the largest dorsal lesions (Moser et al., 1993). A following experiment using ibotenic acid lesions to spare fibers passing through these regions showed that these effects were likely due to impairments in hippocampal processing (Moser et al., 1995). This work suggests that the dorsal hippocampal region is responsible for spatial information processing, and that the ventral hippocampus has comparably little influence on spatial processing and navigation.

The role of the dorsal hippocampus in spatial processing was also shown in other behavioral tasks, including the T-maze and radial arm maze. The T-maze, named for its shape, has one main runway that branches into two runways. A rat is placed at the start of the long runway, then is allowed to make a choice to enter one of the other arms, both of which contain food rewards. After consuming one of the rewards, the animal is placed back at the start location and allowed to make another choice. Choosing the alternate arm results in more reward than returning to the place of the already-consumed reward. In lesion studies investigating the contributions of the dorsal and ventral hippocampus, all groups can perform this task, but when a 5-minute delay is instated between trials, necessitating a longer memory storage about which arm had already been chosen, only the dorsal-lesioned group had an impairment in performance (Hock and Bunsey, 1998; Bannerman et al., 1999, 2002). Additionally, Bannerman et al. found that cytotoxic lesions of the entire hippocampus caused impairment of a non-matching to place task, and that dorsal lesions mimicked this impairment, whereas ventral lesions only caused a slight, insignificant impairment compared to controls (Bannerman et al., 1999). The non-matching to place task takes place in the radial arm maze, consisting of eight equally spaced arms that half of

which are baited with food, where animals are tasked to enter baited arms, avoid un-baited arms, and remember which arms have already been entered to retrieve the food. The performance of rats in these tasks require spatial working or reference memory. When comparing the performance of animals with dorsal, ventral, or complete hippocampal lesions, dorsal-lesioned animals had levels of spatial memory impairment on this task equal to those of the complete hippocampal-lesioned group. In contrast, rats with similarly sized ventral lesions often matched sham controls in performance (Pothuizen et al., 2004).

The dorsal hippocampus's involvement in spatial learning has been demonstrated through inhibition studies and cellular activity. Injections of the $GABA_A$ agonist muscimol into the dorsal hippocampus have a similar effect to dorsal lesions, and decrease time spent in the target quadrant of a water maze (Moser and Moser, 1998). The temporal specificity of hippocampal inhibition by muscimol demonstrates that the dorsal hippocampus is needed at the time of retrieval, not just for learning this task. Analyses of cell activity also confirm high spatial selectivity in the dorsal hippocampus over the ventral hippocampus. For one, differences in place cell activity can be observed between the dorsal and ventral regions. While place cells are present in the CA1 of the ventral hippocampus as well as the dorsal segment, cells in the ventral hippocampus have a smaller number of place fields and spatial selectivity compared to those in the dorsal hippocampus (Jung et al., 1994). A study using c-fos staining as a measure of neuronal activation found that counts of c-fos stained neurons increased all over the hippocampus during a radial arm maze task, but that the dorsal region had a significantly greater increase in activity (Vann et al., 2000). These studies suggest that while the ventral region may still be responsive to spatial input, the dorsal hippocampus has a much greater contribution in these tasks.

1.2.3 Behavioral Regulation

Another area of hippocampal research that is relevant to fear and extinction memory recall is behavioral inhibition and mediating emotional arousal. The hippocampus is suggested to play a role in suppressing inappropriate responses due to evidence that hippocampal damage or inhibition leads to excessive and perseverative responding in tasks where behavior has been extinguished or where the task requires a switching of responses (Gray and McNaughton, 2000; Bannerman et al., 2004). Gray and McNaughton noted that studies using anxiolytics in the septo-hippocampal area produced similar behavioral abnormalities, and proposed the function of a behavioral inhibition system that could become engaged in situations of uncertainty or conflict and influence other brain regions to resolve the conflict (Gray and McNaughton, 2000). In this view, the hippocampus acts as a comparator to recognize when the current state of the environment is not matching what would be expected from memory and may control behavior through projections to other regions such as the hypothalamus and amygdala to elevate arousal, direct attention to alter salience of cues, and suppress behavior. The lack of such a comparator system could explain how lesions of the hippocampus lead to lack of inhibition of behavioral responses when conditions change.

Such a deficit can be seen in mice lacking the GluN1 NMDA receptor subunit of hippocampal pyramidal cells. Mice were trained in a water maze in which two identical beacons were visible above the water, only one of which signaled an escape platform. At the start of the trials, mice were placed either equidistant from the beacons, close to the correct beacon, or close to the incorrect beacon. Although GluN1 KO mice learned to distinguish between two identical beacons and made a similar percentage of correct choices as controls when starting equidistant or close to the correct beacon, they fail to do so if the incorrect beacon is closest to them (Bannerman et al., 2014). If the beacons were not

visually identical, GluN1 KO mice performed at similar levels to controls in all starting locations. This suggests that although GluN1 mice can learn spatial cues to differentiate the locations of the beacons, as seen when both beacons are equidistant, mice with impaired NMDAR plasticity in the hippocampus are unable to use this spatial memory to guide behavior by inhibiting the tendency to approach any such beacon.

Gray and McNaughton proposed a connection between this behavioral inhibition system and anxiety mechanisms, in that the hippocampal system may resolve uncertainty conflicts by increasing the valence of negative associations. Classic anxiolytic drugs like benzodiazepine show similar effects to hippocampal lesions, including reduced hyponeophagia, increased social interaction, quicker latency to cross into a brightly lit compartment, and more time in open arms of an elevated plus maze (Bannerman et al., 2002, 2003). The hippocampus has also been implicated in mediating approach-avoidance conflicts when animals weigh approaching a potentially positive stimulus or avoiding it to prevent negative outcomes. Pharmacological inactivation of the ventral CA1 increases avoidance of an ambiguous cue, whereas inactivation of ventral CA3 increases approach behavior (Schumacher et al., 2018). Work in humans has shown that approach-avoidance conflicts engage the hippocampus (fMRI) and that patients with selective lesions show reduced avoidance behavior in the same task (Bach et al., 2014). Gray and McNaughton suggested that generalized anxiety disorder might similarly involve hyperactivity or hypersensitivity of such a regulator of negative associations and suggested that the effect of classical anxiolytics on anxiety-related behavior could be due to their effects on the hippocampus (Gray and McNaughton, 2000). Human imaging studies have also demonstrated a link between lower hippocampal volume in patients with various forms of emotional dysregulation, including depression, anxiety disorders and PTSD (Campbell et al., 2004; Gilbertson et al., 2010; Irle et al., 2010).

Behavioral inhibition and emotional regulation have largely been attributed to the ventral hippocampus. The anatomical connections of the ventral hippocampus with the hypothalamus and amygdala in particular could be pathways responsible for emotional disruption observed in humans (Campbell et al., 2004; Gilbertson et al., 2010; Irle et al., 2010; Kheirbek et al., 2014), as well as the autonomic effects due to non-specific hippocampal lesions. Indeed, ventral lesions increase the severity of gastric erosion seen in rodents after cold-restraint stress at similar levels to widespread hippocampal lesions, whereas dorsal lesions do not (Henke, 1990). Lesions of the ventral subiculum, a major output pathway for the ventral hippocampus, have also been shown to cause glucocorticoid hypersecretion following restraint stress (Herman et al., 1998). Complete hippocampal lesions have been shown to exacerbate restraint-stress induced gastric ulcer formation. On the other hand electrical stimulation of the ventral hippocampus, which was shown to increase evoked potentials in the lateral central amygdala, has an attenuating effect on ulcer development (Henke, 1990). These findings suggest that the ventral hippocampus exerts some control over autonomic systems, lack of a functional ventral hippocampus may exacerbate stress responses, and activity of this region may decrease stress responses through its projections to various hypothalamic structures.

Similarly, the ventral hippocampus has been related to modulation of anxiety-like behavior. Ventral lesions, but not dorsal lesions, decrease behaviors related to anxiety in ethological behavior tasks. Rodents have natural tendencies to stay in the dark, or travel up against walls (thigmotaxis), ethological behaviors that limit exposure and visibility to predators while foraging (Simon et al., 1993). Importantly, assays such as open field exploration, light/dark shuttle boxes, and elevated plus mazes are sensitive to anxiogenic or anxiolytic drugs known to be effective in humans, where anxiolytics increase time spent in the center of an open field and other such measures (Gray and McNaughton, 2000). In

the light/dark test, rats with ventral lesions were quicker than sham controls to pass from the relatively safe black compartment into the bright, more anxiogenic, white compartment, suggesting reduced stress or anxiety in these animals (Bannerman et al., 2003; McHugh et al., 2004). Hyponeophagia tests, where an impulse to eat food in the middle of an arena is tempered by the novel uncertainty of the arena and food, are also sensitive to ventral lesions, resulting in the lesioned animals having lower latencies to approach and eat the food (Bannerman et al., 2002, 2003; McHugh et al., 2004). Additionally, rats with ventral or complete hippocampal lesions will spend more of their time in the open arms of an elevated plus maze (EPM) compared to rats with dorsal lesions or sham-operated controls, showing a reduced aversion to the exposed arms, another measure of anxiety-like behavior (Bannerman et al., 2002; Kjelstrup et al., 2002). In a successive alley test, designed as an alternate version of the EPM that has progressively more aversive platforms and does not have a center compartment, sham-operated and dorsal-lesioned rats spent most of their time in the first alley, but ventral-lesioned rats spent comparably less time in the first alley and more time in the second alley (McHugh et al., 2004). Rats with ventral hippocampal lesions also have a tendency to defecate less in novel stressful environments (Kjelstrup et al., 2002; Bannerman et al., 2003). These ethological tests of anxiety-like behavior demonstrate that impaired ventral hippocampal processing leads to less anxiety-like behavior, suggesting that the ventral hippocampus plays a role in appropriately connecting anxiety-inducing situations with decreased or inhibited behavior.

The activity of ventral hippocampal neurons also implicates this region in processing stress- and anxiety-inducing stimuli. Using *in vivo* calcium imaging, cells in the ventral CA1 were seen to fire specifically during an animal's activity in the open arms of an EPM (Jimenez et al., 2018). Ventral DG cells also can be stress responsive, firing specifically during attack bouts in a social defeat paradigm or in the anxiogenic center of

an open field (Anacker et al., 2018). Cells have been noted in the basolateral amygdala that respond to either anxiogenic or rewarding stimuli, but these CA1 anxiety cells in the ventral hippocampus project to the lateral hypothalamus, suggesting a pathway for the hippocampus to influence stress and anxiety pathways that bypasses the amygdalar mechanisms of assessing the emotional associations of a cue. A recent study by Biane et al. used calcium imaging to track dorsal and ventral CA1 neurons across an odor learning task to assess how neural encoding changes during the learning of an aversive cue-outcome association (Biane et al., 2023). They found that odors elicited robust responses in dCA1 initially, whereas vCA1 cells responded to the odor after the association was learned, and both populations maintained stable representation after learning. Interestingly, robust responses in CA1 were observed when mice anticipated aversive outcomes under their behavioral control, and not when the outcome was inescapable. The ability of hippocampal cells to preferentially respond to such emotional stimuli suggests not only that the hippocampal representation can activate downstream emotional processing, but that the cells themselves may recognize, or convey information about, emotional valence in a way to influence behavior. Overall, research on the hippocampal role in behavioral inhibition highlights that this region is capable of processing environmental cues, associating these with emotional responses and outcomes, and guiding behavior accordingly.

1.2.4 Contextual Fear Memory in the Hippocampus

Contextual fear conditioning and extinction provide a good model to study hippocampal learning and memory, as learning to associate a context with both fear and safety and mediating defensive responses appropriately involves both spatial memory and behavioral regulation. Generation of a conjunctive representation of the context has been attributed to the dorsal hippocampus given its widespread sensory input and spatial

selectivity. Lesions restricted to the dorsal hippocampus impairs freezing to a conditioned context to a similar degree as entire hippocampal lesions (Maren et al., 1997). Dorsal hippocampal infusion of muscimol has also been shown to impair the context-selectivity of extinction, resulting in similar low freezing in extinction and alternate contexts, suggesting a deficit in contextual memory specifically (Corcoran and Maren, 2001). The activity of dorsal hippocampal neurons reflects broad context encoding during fear recall. In an experiment where groups of rats were either context fear conditioned with an appropriate delay to learn the context-shock association or immediately shocked as controls, fear recall induced Arc mRNA reactivation of dorsal hippocampal neurons in both groups whereas BLA neurons were only reactivated in the fear conditioned group and not the immediate shock group (Zelikowsky et al., 2014). This study suggests that the dorsal hippocampus encodes contextual information used in fear conditioning, whereas the amygdala encodes the fear association specifically. Additionally, optogenetic inhibition of dorsal DG granule cells disrupts the encoding of contextual fear, but does not prevent learning of cued fear association, further demonstrating the specific role in context learning (Kheirbek et al., 2014). While the dorsal hippocampus is largely responsible for spatial selectivity of context memory in the hippocampus, almost all of the studies identifying that place cells remap during fear and extinction and identifying memory ensembles of fear and extinction behavior have been done in the dorsal DG and CA1. These studies show that spatial representations in the dorsal hippocampus are still sensitive to changing valence and can influence fear behavior.

However, the ability to mediate fear behavior is mainly attributed to the ventral hippocampus. The ventral hippocampus is connected to many limbic regions involved in emotional regulation, including the amygdala and prefrontal cortex, crucial regions for expressing and suppressing fear. Ventral hippocampal lesions affect anxiety and fear

behavior generally. Ventral hippocampal lesions have been shown to impair contextual fear conditioning (Bast et al., 2001; McDonald et al., 2018). However, ventral lesions also cause a reduction in freezing responses to unsignalled, and unconditioned, footshocks, and the lesions do not impair spatial processing in a water maze or T-maze (Richmond et al., 1999; Bannerman et al., 2003). This suggests that ventral hippocampal lesions may be influencing the degree of fear responses more than the learned fear association. Optogenetically stimulating or inhibiting ventral DG granule cells during context fear conditioning does not have any significant effect on fear retrieval, although the same stimulation was anxiolytic in the EPM and open field tests (Kheirbek et al., 2014). McDonald et al. have shown that ventral hippocampal lesions impair conditioned inhibitory learning and discriminative conditioning to context when the paired and unpaired contexts have a high degree of cue overlap (McDonald et al., 2018). Ventral hippocampal lesions have also been shown to affect cued fear, when dorsal lesions do not (Maren and Holt, 2004). These studies suggest that the ventral hippocampus may be necessary in ambiguous conditioning situations.

The activity of projecting neurons in ventral CA1 and subiculum underlie the hippocampus's ability to directly mediate fear and extinction recall and behavior. Projections to the amygdala are associated with fear recall. Ventral hippocampal projections to the basal amygdala mediate context conditioned fear, whereas projections to the central amygdala are required for context-dependent retrieval of cued fear memory (Xu et al., 2016). Ventral hippocampal projections to the prefrontal cortex are implicated in control of extinction and fear renewal, fitting with the known roles of the IL and PL. The exact influence of these ventral hippocampal projections is less clear. Brain derived neurotrophic factor (BDNF) has been shown to act in IL to reduce conditioned fear (Rosas-Vidal et al., 2014). Rats that failed to extinguish conditioned fear show reduced BDNF in

ventral hippocampal inputs to IL (Peters et al., 2010). BDNF infusion in the ventral hippocampus additionally increases excitability in the IL, but not PL (Rosas-Vidal et al., 2014). These results suggest that ventral hippocampal activity excites IL and facilitates extinction. However, it has also been demonstrated that hippocampal activity results in feedforward inhibition of the IL to impair extinction recall and promote fear relapse (Marek et al., 2018). This idea fits with other work showing that exciting ventral hippocampal projections to PL attenuates cued fear relapse (Vasquez et al., 2019). It is possible that ventral hippocampal projections to the prefrontal cortex can finely mediate fear activation and suppression through both excitatory and inhibitory influences on IL and PL.

Altogether, contextual fear and extinction memory utilize two different but entwined processes, spatial learning and memory and behavioral regulation. The dorsal hippocampus is highly context selective and supports the encoding and reactivation of contextual memory, the reactivation of which may recruit the ventral hippocampal ability to mediate behavior through projections to other brain regions. These two aspects of hippocampal processing have often been investigated individually, assessing only spatial navigation or only anxiety behavior. However, there is still much to learn about how the hippocampus integrates this information to make quick decisions guided by memory. After context fear extinction, two memories of fear and safety associations exist of the same context. While fear and extinction memory representations have been shown to be distinct in dorsal DG, little is known about how the ventral hippocampus may differentially process this information to activate opposing behavioral responses and influence whether fear or extinction is recalled.

1.3 Overview of Dissertation

The primary goal of my research is to better understand hippocampal mechanisms of contextual fear and extinction learning. It began with the question of how the hippocampus processes opposing memories of fear and extinction, and what might be occurring downstream of dorsal DG ensembles to initiate different behavioral responses. This led to a focus on the ventral hippocampus (vHP), particularly neurons projecting to other brain regions involved in fear and extinction. I hypothesized that vHP projections, specifically those to the IL and BLA mediate fear expression or suppression during fear and extinction recall. Through these experiments I investigate vHP activity to further the knowledge of how the hippocampus contributes to a larger circuit underlying this memory and behavior.

In the first set of experiments (Chapter 2), I investigate whether the activity of vHP projection neurons differs between context fear and extinction recall. I use CTb to retrogradely label vHP projections to the BLA and IL and measure co-localization with the immediate early gene c-Fos to assess activity within these projections. I demonstrate that fear and extinction recall do result in different patterns of activity in vHP neurons. Fear recall causes more c-Fos activation in projections to the BLA compared to projections to the IL, and extinction recall shows the opposite pattern with more c-Fos activation in projections to the IL compared to BLA. These results demonstrate that vHP activity is sensitive to differences in fear and extinction context memory and suggests that differential projection activation may be a mechanism for vHP to influence whether fear or extinction memory is recalled through selective signaling to downstream regions.

In Chapter 3, I sought to test the necessity of vHP projections to IL and BLA in context fear and extinction. I hypothesized that inhibiting vHP projections to BLA would reduce freezing during fear recall but have little to no effect on fear extinction. On the other

hand, I hypothesized that inhibiting vHP projections to the IL would not affect fear recall but would possibly impair recall of extinction and result in higher freezing. I used optogenetic and chemogenetic inhibition to selectively silence these projections during fear recall and extinction recall but did not see any behavioral effects of our manipulations. In Chapter 3 I discuss some of the limitations of these studies and possible interpretations.

In the final set of experiments (Chapter 4), I investigated whether stimulating SST interneurons to increase inhibition within the vHP and IL influenced context fear and extinction memory. I demonstrate that SST interneuron stimulation in the vHP impaired fear recall and lowered freezing. Stimulating SST interneurons in the vHP during fear extinction led to impaired fear learning and increased freezing compared to controls in a stimulation-free test of recall. When stimulating SST interneurons in the IL, no changes in behavior were seen during fear or extinction recall. However, increasing inhibition through this manipulation did replicate findings that IL inhibition during auditory tone extinction impairs extinction learning. These results show that the vHP is necessary for both fear context recall and extinction learning, while IL does not appear to be necessary for context fear or extinction recall but is needed for auditory cue extinction. Altogether, the experiments in this dissertation demonstrate that activity in vHP projection neurons changes between context fear and extinction recall, that vHP activity is necessary for both fear recall and extinction learning, and highlights vHP projections as a future target to further investigate the influence vHP activity has on these behaviors.

Chapter 2: Ventral hippocampal projections to infralimbic cortex and basolateral amygdala are differentially activated by contextual fear and extinction recall[1]

2.1 INTRODUCTION

Fear extinction—repeated exposure to a feared stimulus in a safe environment—is a model of exposure therapy, which is a common and effective treatment for maladaptive fear in patients with PTSD or phobias (Vervliet et al., 2013). A limitation of extinction and exposure therapy is that these procedures involve new learning about the absence of threat, not unlearning of fear, and fear relapse is common (Bouton et al., 2006). How memories of fear and extinction learning interact to modulate expression of emotion is thus a key question with important clinical implications.

Recent studies have identified the hippocampus as a region in which fear and extinction memories may compete for expression. The hippocampal dentate gyrus (DG) contains so-called "fear engram cells," which are neurons active during acquisition of contextual fear that reactivate during recall of contextual fear (Denny et al., 2014). Optogenetic stimulation of these fear engram cells induces the expression of fear, whereas silencing inhibits fear behavior (Liu et al., 2012b; Ramirez et al., 2013). Recent work demonstrates that extinction learning can suppress reactivation of DG fear engram cells and activate a distinct ensemble of cells potentially representing an extinction memory (Lacagnina et al., 2019). Whereas artificial stimulation of the fear memory ensemble increases fear, stimulation of the extinction ensemble reduces fear (Lacagnina et al., 2019). These findings are consistent with the interpretation that fear and extinction memories have

[1] This work was previously published – Brockway ET, Simon S, Drew MR (2023). Ventral hippocampal projections to infralimbic cortex and basolateral amygdala are differentially activated by contextual fear and extinction recall. *Neurobiology of Learning and Memory*. 205:107832. doi: 10.1016/j.nlm.2023.107832.

distinct representations in the hippocampus, and that competition between these representations determines whether fear is suppressed or recovers after extinction. However, little is known about how these distinct ensembles of DG granule cells activate different emotional and behavioral states.

It is believed that hippocampus modulates expression of fear and extinction via projections from CA1 and subiculum in ventral hippocampus (vHP) to the basolateral amygdala (BLA) and prefrontal cortex (PFC). vHP projections to the BLA are believed to provide contextual control of fear in both contextual and auditory cued fear conditioning (Davis, 1992; Herry et al., 2008; Orsini et al., 2011; Jin and Maren, 2015; Xu et al., 2016). The BLA is an essential locus for associative synaptic plasticity during fear learning. Silencing vHP projections to BLA attenuates expression of contextual fear, suggesting that this pathway provides information about the contextual conditioned stimulus (CS) to the amygdala. In contrast, the infralimbic (IL) PFC is implicated in fear extinction learning. Inactivation of IL projections to the amygdala impairs both extinction learning and recall (Milad and Quirk, 2002; Bukalo et al., 2015; Bloodgood et al., 2018). vHP projections to IL appear to enable contextual control of extinction. For instance, in auditory fear conditioning, vHP-IL projections are necessary for context-specificity of fear extinction. vHP projection influence on IL activity is uncertain. Some studies have shown that vHP excites IL and reduces fear, while others suggest that vHP projections potentiate fear through feed forward inhibition of IL (Peters et al., 2010; Rosas-Vidal et al., 2014; Marek et al., 2018). How extinction of context fear influences vHP activity in these pathways is unclear.

While it is clear that vHP projections to IL and BLA can modulate expression of fear and extinction memory, it remains unclear how extinction training affects their activity. Evidence discussed above regarding competing fear and extinction engrams in DG

raises the question whether extinction training orchestrates a corresponding shift in ensemble activity among vHP projection populations. One possibility is that expression of contextual fear and extinction is mediated by the levels of activity in vHP projections to BLA and IL, with vHP-BLA projections favoring expression of contextual fear, and activity of vHP-IL projections favoring suppression of fear after extinction. Alternatively, relative activity in these projections could be invariant across fear and extinction, perhaps because these forms of learning are mediated by changes in synaptic strength in the target regions.

In this study we investigated whether these vHP projections are differentially activated during recall of context fear and extinction memories. We used retrograde tracing to label vHP neurons projecting to the IL and BLA and then assessed c-Fos as a marker of activity within these populations of cells. We show that fear and extinction recall activate vHP projections in opposing manners, with vHP-BLA projections more active during fear expression and IL projections more active during expression of extinction. These results suggest a circuit mechanism through which hippocampal memory representations signal valence and orchestrate appropriate behavioral responses.

2.2 METHODS

2.2.1 Animals

Adult male and female C57BL6/J mice (n=55; Experiment 1: fear (4F, 6M), extinction (5F, 5M), control (3F, 4M); Experiment 2: fear (5F, 5M), extinction (3F, 4M), control (4F, 5M)) approximately 2 months of age were used for all experiments. Mice were housed with littermates in groups of 3-4 in plastic cages with woodchip and paper crinkle bedding and maintained on a 12hr light/dark cycle (7:00-19:00 light on) in a temperature-

and humidity-controlled vivarium. Food and water were provided *ad libitum*. Experiments were conducted during the light phase. Mice were randomly assigned to groups by cage before the start of each experiment. As sex-by-genotype statistical interactions were not present, male and female data were aggregated. All procedures were approved by the University of Texas at Austin Institutional Animal Care and Use Committee.

2.2.2 Surgery

2% isoflurane (1.0 L/min) vaporized in pure oxygen was used to induce anesthesia, followed by 1.5% isoflurane (0.75 L/min) to maintain stable anesthesia after mice were placed in a stereotaxic frame. Cholera Toxin Subunit b (Alexa Fluor 555 and 647 conjugates, Invitrogen) was infused bilaterally (69nL/injection site) using a NanoJect II microinjector (Drummond) targeting the IL (M/L 0.3, A/P +1.8, D/V -2.9) and BLA (M/L 3.45, A/P +-1.65, D/V -4.9), counterbalanced among. Mice were injected subcutaneously with carprofen (10mg/kg), buprenorphine (0.1mg/kg) and buprenorphine XR (3.25mg/kg) in sterile saline (0.9%) to provide analgesia. Subjects were given ~7 days to recover and allow for CTb expression.

2.2.3 Context fear conditioning and extinction

Mice were handled for 2 min per day for 4-5 days prior to behavioral testing. Mice were transported from the vivarium to a holding room adjacent to the test room at least one hour before experimentation. Mice were transported individually to and from the conditioning room in an opaque container. The transport containers were cleaned with a 70% EtOH solution between uses.

Behavioral testing occurred in 30.5 × 24 × 21 cm conditioning chambers (Med Associates), with two aluminum side walls, a Plexiglas door and ceiling, and a white vinyl

back wall. Chambers were contained within a larger, sound-attenuating chamber. An overhead white light illuminated the chamber continuously throughout the procedures. The conditioning chamber contained a straight stainless-steel rod floor (36 rods, spaced 8mm from center to center), was cleaned with a 70% EtOH solution between uses and was scented with 1% acetic acid solution in the waste tray below the floor. Contextual fear conditioning consisted of three 2-sec 0.75mA scrambled foot shocks delivered through the rod floor 160, 240, and 310 seconds after mice were placed in the chamber. Mice were removed 30s after the final foot shock and returned to their home cage.

All behavioral sessions were video recorded at 30 frames/s using a near-infrared camera mounted to the interior door of the chamber. Freezing was defined as the absence of movement, except for those related to breathing. Videos were automatically scored using a linear pixel change algorithm (VideoFreeze; Med Associates). For the fear conditioning session, the percent of time spent freezing was calculated for the 160-sec pre-shock baseline and the 30 sec following each shock. For the extinction and retrieval tests, percent freezing was averaged across the entire 5-min session.

Extinction sessions consisted of 5-min exposures to the original fear conditioning context once per day for 9 days. Subjects in the fear retrieval group stayed in home cages in a holding room during this time. Both extinction and fear retrieval groups were returned to the conditioning chamber for a 5-min retrieval session on the final test day. The control group of mice stayed in their home cage in the holding room during this time.

For context pre-exposure (as shown in Fig. 3) mice were placed in the conditioning chamber for 5-minute exposure sessions once per day for 9 days, so that mice received the same amount of context exposure as mice receiving extinction training. Mice in the pre-exposure group were fear conditioned the day following the final pre-exposure and received a retrieval test a day later.

2.2.4 Immunohistochemistry

Ninety minutes after the retrieval test, mice were deeply anesthetized with ketamine/xylazine (150/15 mg/kg) and transcardially perfused with 0.01M phosphate buffered saline (1x PBS), followed by 4% paraformaldehyde (PFA) in 1× PBS. Brains were extracted and post-fixed overnight at 4°C in 4% PFA and then transferred to a 30% sucrose in 1× PBS at 4°C for two days. 35 μm coronal sections were collected on a cryostat. For immunohistochemistry, sections were washed in 1× PBS and blocked at room temperature (RT) for 1.5 h in 5% normal donkey serum (NDS) in 1× PBS with 0.5% Triton-X (PBS-T). Sections were incubated with primary antibodies (1:2,000 rabbit anti-c-Fos polyclonal antibody, Synaptic Systems #226-003) diluted in 5% NDS in PBS-T overnight at room temperature. Sections were rinsed in 1× PBS-T and incubated in secondary antibodies (1:500 Alexa Fluor 488-conjugated AffiniPure Donkey Anti-rabbit IgG, Jackson ImmunoResearch Laboratories) in 1× PBS-T for 2 h at RT. Sections were washed in 1× PBS, mounted onto slides, and stained with 1:1000 DAPI for 5 minutes before being coverslipped with Fluoromount-G (SouthernBiotech).

2.2.5 Imaging and quantification

4-6 z-stacks per animal were taken of the ventral hippocampus on a Zeiss Axio Imager.M2 microscope with Apotome.2 and StereoInvestigator 64 software using an ECPlan-Neofluor 20x objective. Images were taken within -2.9 to -3.8 mm bregma, spanning a range of ventral CA1 up to the distal CA1/subiculum border. Fluorescent channels were separated in ImageJ to manually count CTb-labeled neurons, using the multi-point tool to mark and save pixel coordinates as region of interest (ROI) sets to compare between channels. A cell was considered positive for CTb labeling if a clear halo of fluorescent labeling could be identified surrounding the soma location as defined by

DAPI staining. A cell was considered positive for c-Fos labeling if the fluorescence level in the soma was 2x background levels of fluorescence. Fluorescence was measured in ImageJ as the mean fluorescent intensity within a selected region of interest drawn around the soma, and the background was considered the mean fluorescent intensity of a region of surrounding tissue of uniform intensity and no visibly labeled cells. Cell counts were averaged for each subject and are displayed as means ±SEM.

2.2.6 Statistical analysis

Data were analyzed using two-sided t-tests or ANOVA, using repeated measures when appropriate. Significant ANOVAs were followed by post hoc Holm-Sidak's test for multiple comparisons. Data analysis was performed on Prism (GraphPad Software) or JMP (SAS Institute). The α value was set at 0.05 for all analyses. All data are presented as mean ±SEM.

2.3 RESULTS

2.3.1 Fear and Extinction retrieval activate different ventral hippocampal projections

To label hippocampal projection neurons, male and female C57BL/6J mice were injected with the retrograde tracer Cholera Toxin subunit B (conjugated to Alexa Fluor 566 and 648), with injections targeted to IL and BLA, counterbalanced by fluorophore across mice (Fig. 1A-B). A week later, mice were divided into three groups: fear, extinction, and home cage control. Mice in all groups were context fear conditioned, with no differences in freezing between groups (Fig. 1C; two-way RM ANOVA; no interaction effect, $F_{(6,72)}$ = 0.099, p = 0.996; effect of time, $F_{(3,72)}$ = 40.510, p <0.001; no effect of group, $F_{(2,24)}$ = 0.258, p = 0.775). Mice in the Extinction condition then received 9 days of extinction

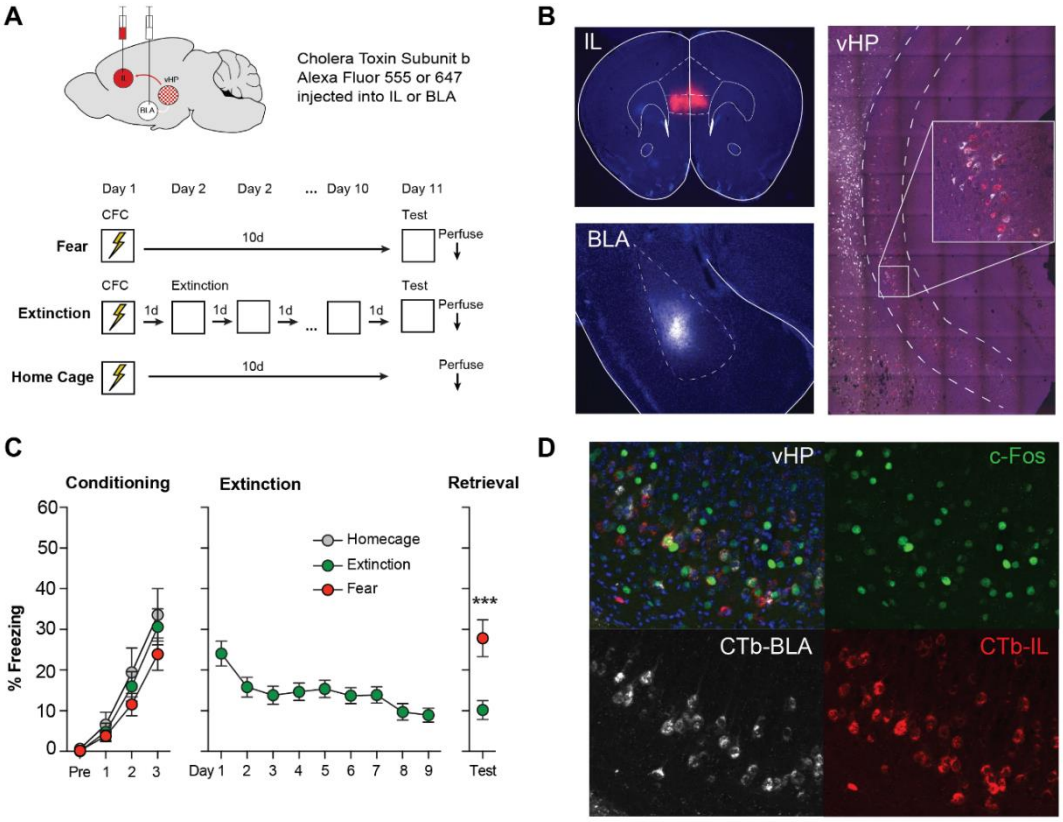

Figure 2.1: Labeling of ventral hippocampal projections in CFC and Extinction.

(A) Experimental design and timeline. (B) Representative images of CTb injections in IL and BLA, with cell bodies labeled in vHP. (C) Freezing behavior during CFC, Extinction, and Retrieval sessions. The left panel depicts freezing during the pre-shock period and post-shock intervals of the conditioning session. The middle and right panels display mean freezing during each 5-min extinction or retrieval session. During extinction, freezing decreased across sessions. In the retrieval test, the fear group exhibited higher freezing than the extinction group. (D) Representative images of c-Fos-labeled vHP neurons and overlap with CTB as imaged for quantification.

training, followed by a test for recall of extinction on the final day. Mice in the Fear group did not receive extinction and were tested for recall of fear on the final test day. Mice in the home cage control group did not receive any further testing. Freezing to the conditioning context decreased across days for the extinction group (One-way RM

ANOVA, $F_{(8,72)} = 4.341$, $p < 0.001$), and freezing was significantly higher during retrieval testing for the fear group than the extinction group ($t_{(19)} = 2.942$, $p = 0.008$). Mice were perfused 90 min after the final session (or taken from their home cage for the control), and immunohistochemistry against c-Fos was used to assess activity within vHP neurons. We predicted that mice in the Fear group would display more c-Fos activity in BLA-projecting vHP neurons than in IL-projecting neurons, whereas Extinction mice would exhibit the opposite pattern.

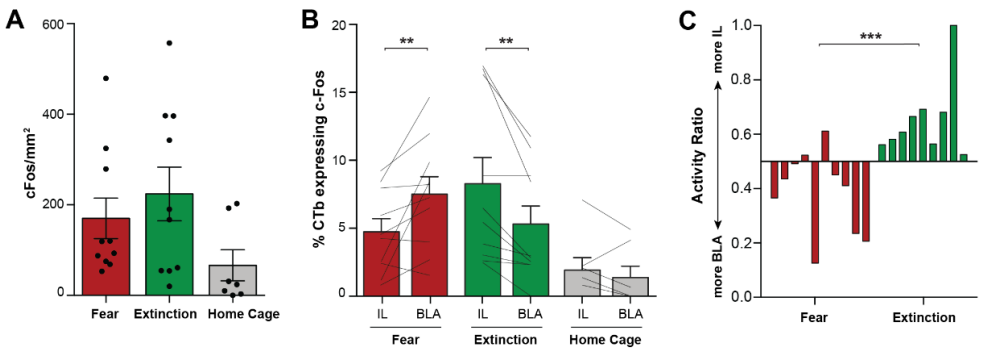

Figure 2.2: Fear and Extinction retrieval activate different ventral hippocampal projections.

(A) Fear and Extinction retrieval groups exhibited similar overall c-Fos density in the ventral hippocampus, and c-Fos density trended lower in the home cage control group. (B) Percentage of IL-projecting and BLA-projecting CTb-labeled neurons that were positive for c-Fos. (C) A projection activity ratio was calculated for each subject (each bar represents one mouse). The ratio was calculated as the percentage of IL-CTb labeled cells positive for c-Fos as a function of the percentage of all CTb labeled cells positive for c-Fos.

The overall density of c-Fos-positive cells did not differ among the extinction, fear, and home cage groups, although the home cage level was numerically lower and the ANOVA bordered on significance (Fig. 2A; ANOVA, $F_{(2,24)} = 3.248$, $p = 0.056$). Activity

within specific projection populations was measured as the percentage of CTb-positive neurons that co-expressed c-Fos (Fig. 1D). A repeated-measures ANOVA revealed a significant Projection X Behavior Group interaction (Fig. 2B; $F_{(2,24)} = 12.33$, $p < 0.001$). Extinction mice displayed more c-Fos expression in IL-projecting neurons compared to BLA-projecting neurons, whereas Fear mice had more c-Fos expression in BLA projections compared to IL projections (Holm-Sidak's test, Ext: $p = 0.004$; Fear: $p = 0.007$). We additionally computed a within-subjects measure comparing the relative levels of IL and BLA projection activity, which was the percentage of IL-projecting cells that are positive for c-Fos divided by the overall percentage of IL- and BLA-projecting cells that were c-Fos positive (Fig. 2C). This metric confirmed that fear and extinction recall activate these vHP projection pathways with different strengths: there was higher c-Fos expression in BLA projections compared to IL projections during fear retrieval, whereas this balance was shifted towards more c-Fos expression in IL-projecting neurons during extinction retrieval ($t_{(18)} = 3.815$, $p = 0.001$).

2.3.2 Effect of context exposure on projection activity

Although our results are consistent with the idea that fear and extinction differentially recruit hippocampal output pathways, there is the alternative possibility that ventral hippocampal activity is shaped by the amount of context exposure rather than by extinction *per se*. In the prior experiment, the extinction group received 9 more days of exposure to the context than the fear recall group, raising the possibility that context exposure, rather than extinction, shifted the balance of vHP projection activity. To control for the amount of context exposure between groups, we conducted a second behavioral

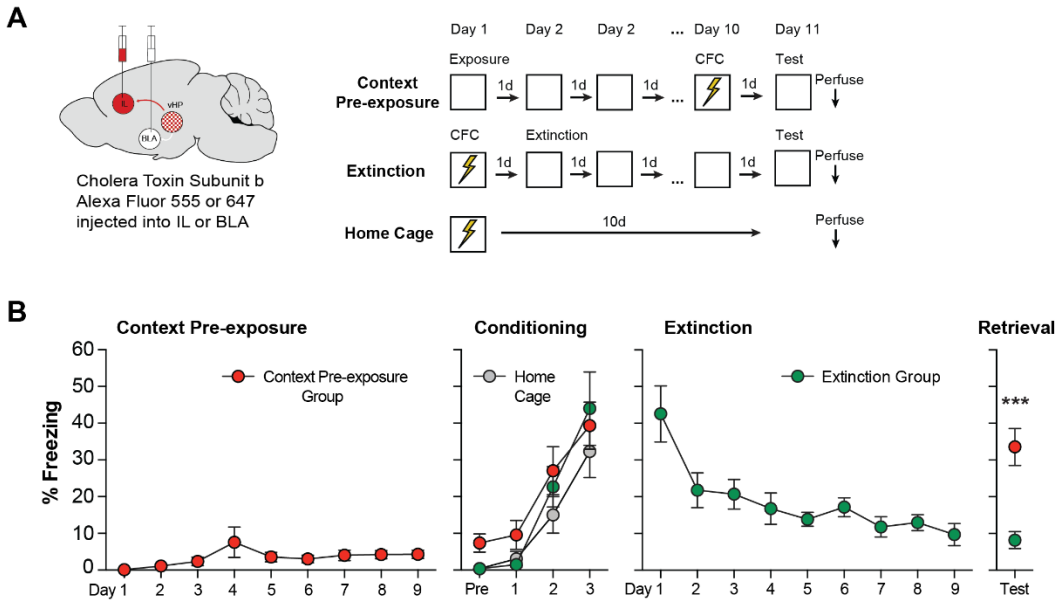

Figure 2.3: Fear conditioning and extinction in groups with equivalent context exposure

(A) Experimental design and timeline. (B) Freezing during context preexposure, conditioning, extinction and recall. The fear recall group exhibited significantly higher freezing than the extinction group during retrieval.

experiment in which the fear and extinction groups received the same total amount of context exposure. As before, CTb was used to label vHP projections to IL and BLA. One group of mice (pre-exposure group, Fig. 3) then received 9 sessions of pre-exposure to the context prior to context fear conditioning, followed by a fear retrieval session. The extinction group was treated as before: mice were context fear conditioned (without pre-exposure), then given 9 days of extinction and an extinction retrieval test. Home cage control mice were context fear conditioned and later taken straight from their home cage for perfusion. In this design, mice in the fear and extinction recall groups had the same amount of exposure to the conditioning context.

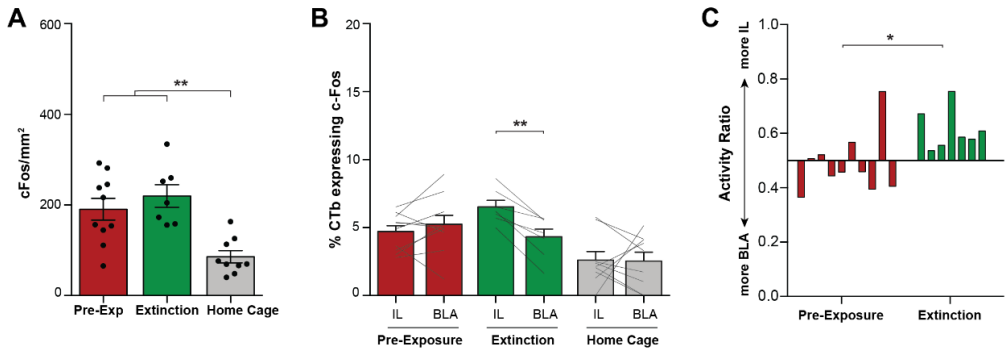

Figure 2.4: Fear and extinction recall activate different vHP projections even with cumulative context exposure is equated.

(A) Overall c-Fos density in vHP. Density of Extinction and Fear groups exceeded that of the Home Cage group. (B) Percentage of IL-projecting and BLA-projecting CTB+ neurons expressing c-Fos. The extinction group showed higher c-Fos expression in IL projections than BLA projections. In the fear retrieval and home cage groups, levels of cFos expression did not differ by projection. (C) Projection activity ratio for each subject.

The pre-exposed group of mice exhibited low levels of freezing during pre-exposure sessions. All mice showed evidence of fear acquisition during conditioning, with freezing increasing across the 3 footshock presentations and no differences between groups (Fig. 3b; two-way RM ANOVA; no interaction effect, $F_{(6,69)} = 0.478$, $p = 0.823$; effect of time, $F_{(3,69)} = 44.63$, $p < 0.001$; no effect of group, $F_{(2,23)} = 1.350$, $p = 0.279$). Freezing decreased across trials during extinction for the extinction group of mice (One-way RM ANOVA, $F(8,48) = 8.613$, $p < 0.001$). In the retrieval test, the fear group of mice froze significantly more than the mice that received extinction training ($t_{(15)} = 4.422$, $p < 0.001$). Mice were perfused 90 min after the retrieval session (or taken from their home cage for the control) and immunohistochemistry against c-Fos was used to assess activity within vHP neurons as before.

The extinction and pre-exposure groups showed similar densities of c-Fos labeling in the vHP; c-Fos density showed comparatively lower activity in the home cage control group (Fig. 4a; $F_{(2,23)}$ =10.710, $p < 0.001$; Holm-Sidak's, Ext vs Fear $p = 0.358$, Ext vs HC $p = 0.001$, Fear vs HC $p = 0.003$). Like the previous experiment, the extinction group displayed more c-Fos expression in IL-projecting neurons compared to BLA-projecting neurons (Fig. 4b). In contrast, the pre-exposed group exhibited similar levels of c-Fos expression in IL and BLA projections during fear retrieval. A two-way repeated measures ANOVA revealed a significant Projection X Behavior Group interaction ($F_{(2,23)} = 4.699$, $p = 0.019$). A Holm-Sidak post hoc test confirmed that the extinction group had higher activity in the IL-projecting than BLA-projecting neurons, whereas in the pre-exposure group activity was not significantly different between the two populations (Ext: $p = 0.014$, Pre-exp: $p = 0.610$). This pattern was also observed in the activity ratio (Fig. 4c), where the extinction group ratios skew higher, closer to the IL end of the scale, whereas the ratios for the pre-exposure group are centered on 0.5, indicating comparable percentages of c-Fos activation within vHP-IL and vHP-BLA projections (Effect of Behavior Group: $t_{(15)} = 2.576$, $p = 0.021$). These results replicate our previous finding that extinction recall is associated with proportionally more activation of vHP cells projecting to IL than in those projecting to BLA. Differing from the prior experiment, the fear recall group that was pre-exposed to the context had comparable amounts of c-Fos activation in both projection populations.

Lastly, we looked for a relationship between freezing behavior and the relative activation of vHP projections. Combining data from both experiments (excluding home cage mice), we observed a significant inverse correlation between the projection activity ratio (as described above) and freezing behavior ($r^2 = 0.141$, $p = 0.023$). Low freezing was

associated with a high ratio, reflecting more activity in IL-projecting neurons, whereas high freezing was associated with relatively greater activity in BLA- projecting neurons.

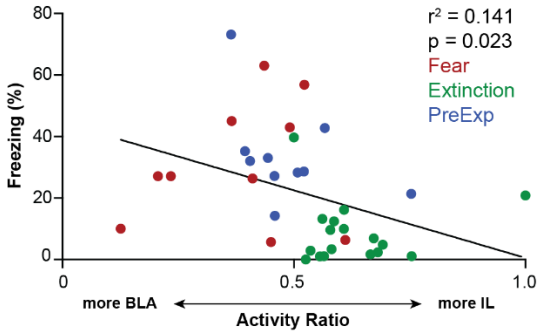

Figure 2.5: Projection activity ratio correlates with freezing.

Linear regression of activity ratio plotted against percent of time spent freezing during the recall test for both experiments.

2.4 Discussion

We investigated the role of vHP projections to BLA and IL in expression of fear and fear extinction memories. Previous research has shown that the hippocampus generates context fear and extinction memory representations that give rise to opposing behavioral responses to a shared context. The current study addresses the circuit mechanisms through which these hippocampal representations might influence behavior. Our results demonstrate that recall of context fear and extinction differentially activate ventral hippocampal projections to the amygdala and IL. Although both projections are active at both retrieval tests, the relative levels of activity shift. Fear recall was associated with more c-Fos expression in vHP cells projecting to the BLA than in cell projecting to IL, whereas extinction recall was associated with more c-Fos activity in cells projecting to the IL than those projecting to BLA. This differential activation may provide a mechanism for the

hippocampus to influence downstream regions involved in fear processing by weighting system level-activity more towards fear activation in the amygdala or extinction-related processing in the IL.

Previous studies of hippocampal memory have shown that the hippocampus encodes emotional valence in addition to contextual components of episodic memory. Learning new valence associations in a stable environment, such as fear conditioning to a previously neutral environment or extinction of context fear, causes remapping in place cells as well as population-level changes in ensemble activity. Place fields of CA1 cells are typically stable over time in the same environment; however, when the valence of an environment changes because of fear conditioning or extinction, place fields remap, perhaps forming new representations of the same context (Moita, 2004; Wang et al., 2012, 2015). Activity-dependent tagging studies support this idea. Extinction learning suppresses reactivation of DG fear ensembles and activates a distinct population of neurons potentially representing the extinction memory (Lacagnina et al., 2019). These studies show that hippocampal coding of context memory is sensitive to changing valence within a stable context.

The current study suggests a mechanism through which putative fear and extinction representations in the hippocampus might influence behavior. The hippocampus may activate context fear expression through preferential activation of projections to BLA, which is consistent with other work investigating hippocampal-amygdala interactions. The BLA is necessary for acquisition and expression of fear conditioning for both contextual and tone conditioned stimuli (Davis, 1992; Fanselow and Ledoux, 1999). Hippocampal projections to the amygdala are believed to convey context memory representations for contextual fear conditioning and contextual regulation of cued fear (Herry et al., 2008; Orsini et al., 2011; Jin and Maren, 2015; Xu et al., 2016). Disrupting activity in this

projection, through optogenetic inhibition or stimulation, impairs encoding and retrieval of contextual fear (Xu et al., 2016; Jimenez et al., 2018; Graham et al., 2021). As the current study focused on activity within the vHP, we cannot determine whether the projections we assessed target excitatory or inhibitory neurons or their overall influence on BLA processing. However, our finding that context fear recall is associated with more c-Fos expression in ventral hippocampal projections to the amygdala, rather than IL, is consistent with the idea that preferential activation of the hippocampus-to-amygdala pathway initiates recall of contextual fear responses.

On the other hand, extinction recall was associated with relatively more c-Fos expression in neurons projecting to the IL, a region important for extinction learning and recall (Quirk et al., 2000; Milad and Quirk, 2002; Laurent and Westbrook, 2009; Bukalo et al., 2015; Do-Monte et al., 2015; Bloodgood et al., 2018). While the IL is well known for its role in extinction, the function of vHP projections to this region is less clear. Activity in vHP-IL projections can both increase and decrease fear expression, perhaps because the projections can directly excite IL principal cells and elicit feed-forward inhibition through interneurons (Gabbott et al., 2002; Hoover and Vertes, 2007; Liu and Carter, 2018). Marek et al. recently demonstrated that activity of this projection evokes feed-forward inhibition of IL principal neurons through parvalbumin-expressing interneurons. In addition, they showed that pharmacogenetic activation of vHP-IL projections promotes cued-fear recall whereas inhibition diminishes fear renewal (Marek et al., 2018), suggesting that activity in this pathway increases fear expression. However, there is also evidence that strengthening of vHP projections to IL supports extinction learning. NMDA receptor currents at vHP-IL synapses are reduced after fear conditioning, and extinction was shown to reverse this effect (Soler-Cedeño et al., 2019). Furthermore, brain derived neurotrophic factor (BDNF), of which release in IL is both necessary and sufficient for extinction, is elevated in vHP

following extinction. BDNF infused into the vHP enhances IL firing rates, and BDNF levels in vHP inputs to IL are reduced in rats that fail to extinguish fear (Peters et al., 2010; Rosas-Vidal et al., 2014). These findings suggest that vHP-provided BDNF is necessary for extinction-induced plasticity in IL. Taken together, the vHP appears able to bidirectionally modulate IL excitability and thus may exert fine control over extinction memory recall. In our experiments we observed higher activity in IL-projecting neurons in the extinction recall group, which fits with a model in which vHP-IL activity promotes extinction, potentially by exciting IL principal neurons. However, IL projection activity was not absent in our fear recall condition, and we did not assess the cellular targets in IL of these projections.

Importantly, our studies demonstrate that the increase in vHP-IL projection activity during extinction recall was not caused by mere context exposure. Our second experiment included pre-exposure to the context prior to fear conditioning in the fear recall group to control for potential effects of context exposure. Even when the amount of context exposure was equivalent between the fear recall and extinction groups, the relative activity of vHP-IL and vHP-BLA projections differed between groups. The extinction group displayed more activation of vHP-IL-projections than vHP-BLA projections, whereas in the fear recall group the two projections were equally active. It is important to note that because the total amount of context exposure was equated, the time between conditioning and the retrieval test was shorter for the pre-exposure group. It is possible that fear conditioning only a day prior to test could influence the balance of projection activity—for instance, heightening vHP-BLA activity or dampening vHP-IL recruitment from pre-exposure. That the pre-exposed fear recall group did not show increased vHP-BLA projection (in contrast with the non-pre-exposed group in the first experiment), suggests that pre-exposure to the context prior to conditioning suppresses recruitment of vHP-BLA

projections during later retrieval. An intriguing possibility is that vHP-BLA projections are recruited most strongly when the training history endows a CS unambiguous valence, whereas ambiguous cues (such as those that have been conditioned and then extinguished, or pre-exposed and then conditioned) recruit more distributed circuit activity.

Alternately, outcomes in the pre-exposure group could reflect latent inhibition, wherein prior exposure to the conditioned stimulus (CS) weakens its associability during acquisition of the CS-shock association, leading to less learned fear (LeDoux, 2014; Miller et al., 2022). Whether latent inhibition actually occurred is unclear. Context pre-exposure had no effect on context acquisition and, during the recall test, mice in the pre-exposure group froze at levels comparable to mice that did not receive pre-exposure. However, such comparisons are to be interpreted with caution because they are made across experiments and across groups with different acquisition-to-test intervals. Thus, we cannot rule out an effect of latent inhibition in this experiment. Indeed, it is possible that the relatively equal activity in BLA- and IL-projecting cells in the pre-exposed group reflect a weaker fear association. It is further possible that context pre-exposure recruits mechanisms similar to those recruited by extinction. Manipulations to ventral hippocampus, BLA, and IL all influence latent inhibition and extinction, and both latent inhibition and extinction of tone-shock associations are context dependent (Miller et al., 2022). A prominent theory of latent inhibition is that competing context-US and context-noUS memories are retrieved during testing, with the CS-noUS association dominating (Miller et al., 1986; Bouton, 1993). This theory of latent inhibition resembles our view of extinction as establishing a memory that competes with the original fear association. Perhaps under experimental conditions that promote stronger latent inhibition, patterns of activity in vHP would more closely resemble those exhibited during extinction.

Thus far, we have interpreted the vHP projection activity as controlling expression of fear and extinction. It is equally possible, however, that vHP activity is associated with behavioral correlates of fear and extinction, rather than fear and extinction *per se*. An example is exploratory behavior, which tends to correlate inversely with freezing. It is possible that vHP-IL activity promotes exploration (or vice versa). If so, then similar patterns of vHP-IL activity should be observed with other behavioral manipulations that modulate exploration. For example, exposure to a novel context, which typically evokes exploration, might increase activity in vHP-IL projections. Our data illustrate that expression of fear and extinction are associated with shifting patterns of activity within vHP-IL and vHP-BLA projections, but further experimentation will be required to understand the causal significance of these shifts with respect to behavior and mental processing.

This study focused on vHP projections to BLA and IL because of the abundant evidence linking these two target regions to conditioned fear acquisition and expression. However, it is likely that other hippocampal projections modulate these forms of learning and behavior. For instance, vHP projections to the lateral septum are involved in exploratory behavior and may help differentiate extinction and exploration (Trent and Menard, 2010). Prelimbic cortex (PL) activity increases during freezing behavior to a conditioned auditory tone, and PL neurons projecting to BLA have increased c-Fos activity after fear renewal compared to extinction (Burgos-Robles et al., 2009; Orsini et al., 2011). vHP projections have a direct role in this activity, as contralateral lesions to disrupt vHP-PL connectivity eliminate fear renewal (Orsini et al., 2011). Additionally, the IL and PL mutually inhibit each other, with vHP inputs preferentially driving the relevant corticocortical neurons (Liu and Carter, 2018). Disparity in IL and PL firing correlates with auditory fear expression, with high freezing animals having higher PL firing rates

compared to IL rates (Giustino et al., 2016). This finding leads us to speculate that the relative activity in hippocampal projections to PL and IL shifts between fear and extinction, with extinction recall potentially activating a larger percentage of vHP-IL projections and fear recall associated with more vHP-PL projection activity. Ventral hippocampal projections can also both excite and inhibit IL and PL, allowing for further modulation of the balance of activity in these regions. Hippocampal projections to the nucleus accumbens (NAc) are also a potential target for future studies. The NAc is known for its role in reward processing, latent inhibition, and activity in vHP-NAc projections is necessary for conditioned place preference (LeGates et al., 2019; Miller et al., 2022). It is conceivable that the reduction of fear during extinction is associated with increased activity in the vHP-NAc pathways, coinciding with the re-emergence of appetitive behaviors such as foraging.

In conclusion, we have shown that pyramidal neurons in the ventral hippocampus that project to the IL and BLA exhibit different patterns of c-Fos activation during fear or extinction recall. Although these vHP projections are active during both fear recall and extinction recall, extinction recall is associated with more c-Fos expression in vHP cells projecting to IL compared to those projecting to BLA, whereas fear recall is associated with more c-Fos expression in vHP-BLA projections. These results support the view that hippocampal representations for a context evolve in conjunction with changes in the context valence, and this evolution may provide a mechanism for orchestrating appropriate behavioral responses through selective activation of projection pathways.

Chapter 3: Inhibition of Ventral Hippocampal Projections

3.1 INTRODUCTION

The hippocampus generates contextual representations, a process that is integral to context fear conditioning and contextual guidance of cued fear. Lesioning the hippocampus shortly after auditory fear conditioning greatly reduces freezing to the fear context but not to a learned tone (Kim and Fanselow, 1992; Maren et al., 1997). It is thought that the hippocampus facilitates the rapid learning of a configural representation of the environment, although it is not necessary for learned associations to discrete cues or recall of more remote memories, and context fear learning can still occur in lesioned animals through compensatory mechanisms following extensive training (Fanselow, 2010). In addition to facilitating context fear memory, the hippocampus mediates the context-specific expression of extinction. Rats that are auditory fear conditioned then extinguished in a separate context will show low freezing to the CS in the extinction context, but high freezing in the original conditioning context. Inactivation of the dorsal hippocampus with infusion of the GABA receptor agonist muscimol disrupts this context-specific recall of extinction, resulting in low freezing in both contexts (Corcoran and Maren, 2001). Furthermore, ensembles of neurons in the hippocampal DG that are active during encoding of fear or extinction are both necessary and sufficient for fear and extinction recall (Liu et al., 2012a; Ramirez et al., 2013; Denny et al., 2014; Lacagnina et al., 2019). These studies demonstrate that the hippocampus is responsible for contextual processing underlying fear and extinction memory. However, how the hippocampus conveys contextual information to influence fear behavior is less understood.

We hypothesize that ventral hippocampal (vHP) neurons convey information about context memory and associated fear through selective signaling of projection neurons to

other fear and extinction related brain regions. In the previous chapter we demonstrated that vHP neurons projecting to the IL and BLA have different patterns of activity during context fear and extinction recall. These results are a snapshot in time, identifying active neurons during these two recall points. Although we now know that fear recall causes more c-Fos activity in vHP neurons projecting to BLA than to IL, and extinction recall causes more c-Fos activity in IL projecting neurons than BLA, this data cannot determine what this activity may be doing to influence fear behavior. Selectively targeting vHP projections to the IL and BLA and manipulating their activity during the recall tests is one way to investigate if this activity is necessary for fear and extinction recall in the manner suggested in our previous experiments.

The vHP projection to BLA has largely been studied for its influence on fear recall. It is well accepted that the hippocampus contributes contextual information to the amygdala during fear conditioning (Maren et al., 2013). Previous research has indeed confirmed that the vHP projection to the basal amygdala (BA) specifically is necessary for context fear recall. Xu et al. have previously targeted vHP->BA using a retrograde RabiesΔG-ArchT virus injected in the BA and optic fibers placed above the ventral CA1 (Xu et al., 2016). They report that inhibiting this projection during context fear retrieval decreased freezing behavior. Jimenez et al. have shown optogenetic stimulation of this pathway, injecting ChR2 into the vHP and the optic fiber above the terminals in the BA (Jimenez et al., 2018). They find that stimulating at 10 Hz during either fear conditioning or a recall test session impaired context fear recall, which they attribute to disrupted signaling in this pathway. It has been shown that high frequency stimulation (20 Hz) of vHP->BA induces feed-forward inhibition of the amygdala and impairs context fear, whereas 4 Hz stimulation excited BA neurons, did not impact expression of context fear, and increased fear generalization (Graham et al., 2021). These studies demonstrate that

disrupting or inhibiting activity of vHP projections to the BA impairs fear recall, whereas low frequency stimulation increases fear.

The role of the vHP projection to the IL is less well understood. In Chapter 2 we identify that there is higher activity in vHP neurons projecting to IL than BLA during extinction recall. This suggests that the vHP-IL pathway is engaged during fear extinction recall. Work out of the Quirk lab supports this, showing that BDNF from hippocampal terminals excites IL and promotes extinction (Peters et al., 2010; Rosas-Vidal et al., 2014). It has also been shown that NMDA receptor currents at vHP->IL synapses are reduced following auditory fear acquisition, and extinction reverses this effect, further suggesting that plasticity along this pathway is associated with fear suppression and extinction (Soler-Cedeño et al., 2019). However, Marek et al. have demonstrated that optogenetic stimulation of vHP recruits strong feed-forward inhibition in the IL, and chemogenetic inhibition of vHP->IL impaired context renewal of cued fear (Marek et al., 2018). They attribute this effect to feed-forward inhibition in layer 2/3 by PV interneurons. Liu and Carter have shown that hippocampal inputs target both layer 2/3 and layer 5 in the IL, but are most effective at driving action potential firing of cortico-cortical neurons in layer 5 (Liu and Carter, 2018). It is possible that the opposing behavioral effects on fear expression and suppression seen in the above studies reflect differences in post-synaptic targets or signaling patterns, and there is much to still learn about the role of this projection in fear extinction. Additionally, all the work mentioned here used auditory cue conditioning. Given that the vHP projections to the BLA display different activity patterns in context conditioning and cued conditioning, the influence of vHP projections to IL in context fear extinction should be further examined.

Here I will describe multiple experiments aimed at specifically inhibiting the vHP projections to IL and BLA to test if they are necessary for context fear and extinction recall

in the manner suggested by our c-Fos activity experiment. The first experiment targets the vHP->IL projection using an optogenetic approach. In Chapter 2 we saw more c-Fos activation in vHP projections to the IL compared to BLA during extinction retrieval. Thus, we hypothesized that inhibiting this pathway during fear extinction recall might inhibit recall and increase fear. The second experiment targets the vHP->BLA projection using a chemogenetic approach. In Chapter 2 we found that fear recall was associated with relatively more c-Fos activity in vHP projections to BLA than IL, and based on the above studies we expect that inhibiting this pathway would reduce freezing during fear recall. However, the BLA has been shown to encode extinction memory as well as fear (Herry et al., 2006, 2008; Pape and Pare, 2010), and how activity in this projection might affect freezing behavior during extinction recall is unknown. Our c-Fos activity data showed activation of vHP projections to BLA and IL during both fear and extinction recall, although the relative amount of activity in the two populations changed. It's possible that inhibiting the non-dominant projections during recall could facilitate fear or extinction recall by further shifting this balance of activity. We hypothesized that inhibiting BLA projections during extinction recall or IL projections during fear recall would not have a noticeable effect on behavior but wanted to directly test this. As such, we sought to investigate the necessity of these projections during both fear recall and extinction recall timepoints.

3.2 METHODS

3.2.1 Animals

Adult male and female C57BL6/J mice (Experiment 1: n=16 (8M, 8F), Experiment 2: n=15 (9M, 6F) mice approximately 2-4 months of age were used for all experiments.

Mice were housed with littermates in groups of 3-4 in plastic cages with woodchip and paper crinkle bedding and maintained on a 12hr light/dark cycle (7:00-19:00 light on) in a temperature- and humidity-controlled vivarium. Food and water were provided ad libitum. Experiments were conducted during the light phase. Mice were randomly assigned to groups by cage before the start of each experiment. As sex by genotype statistical interactions were not present, male and female data were aggregated. All procedures were approved by the University of Texas at Austin Institutional Animal Care and Use Committee.

3.2.2 Viruses

Adeno-Associated Viruses (AAVs) SE771:AAV(2-retro)-CAG-Cre, SE761:AAV(1)-hSyn-tdTomato(Cre) and SE334:AAV(1)-hSyn-ArchT(Cre) were made at UT Austin in the lab of Boris Zemelman. pAAV-hSyn-DIO-hM4D(Gi)-mCherry was acquired from Addgene (Addgene viral prep #44362-AAV8, RRID:Addgene_44362, https://www.addgene.org/44362/).

3.2.3 Surgery

2% isoflurane (1.0 L/min) vaporized in pure oxygen was used to induce anesthesia, followed by 1.5% isoflurane (0.75 L/min) to maintain stable anesthesia after mice were placed in a stereotaxic frame. For optogenetic inhibition, SE771:AAV(2-retro)-CAG-Cre was infused bilaterally using a NanoJect II microinjector (Drummond) into the IL (25nl/injection, M/L +/- 0.3, A/P +1.8, D/V -2.9) and SE761:AAV(1)-hSyn-tdTomato(Cre) or a mixture of SE761:AAV(1)-hSyn-tdTomato(Cre) and SE334:AAV(1)-hSyn-ArchT(Cre) in a 1:5 ratio targeting the vHP (50nl/injection, M/L +/-3.5, A/P -3.2, D/V -3.4 and -3.8). DREADDs manipulation in Experiment 2 used SE771:AAV(2-retro)-CAG-Cre

injected into the BLA ( ), and either SE761:AAV(1)-hSyn-tdTomato(Cre) or pAAV-hSyn-DIO-hM4D(Gi)-mCherry injected into vHP (50nl/injection, M/L +/-3.5, A/P -3.2, D/V -3.4 and -3.8). Optogenetic fibers (0.22 NA, 200µm core, constructed in lab (Sparta et al., 2012)) were implanted bilaterally 200µm above the injection sites, using the same coordinates for vHP (3.5mm fiber, D/V -3.5). The fibers were secured in place with a layer of dental acrylic. Mice were injected subcutaneously with carprofen (10mg/kg), and buprenorphine XR (3.25mg/kg) in sterile saline (0.9%) to provide analgesia. Subjects were given ~21 days to recover and allow for viral expression.

3.2.4 Optogenetics

Cranial implants were bilaterally connected via fiber optic patch cables to a light source interfaced with a FC/PC rotary joint (Doric Lenses). ArchT stimulation was delivered with a continuously delivered 532 nm laser (Shanghai Dream) with an intensity of 11 mW at the end of the fiber optic implant.

3.2.5 Context fear conditioning

Prior to testing mice were handled to habituate to the experimenter. For both experiments, mice were gently handled for 2 min per day for the first two days. For optogenetics, the third day mice were put in a gentle restraint and fibers were cleaned. On days 4-6 mice were gently restrained, attached to dummy patch cables, and allowed to explore a clean cage with familiar bedding to build familiarity to being tethered to optogenetic patch cables. For chemogenetics, mice were handled on day 3 and 4 and restrained by the scruff to habituate to the process of being handled for i.p. injections.

Behavioral testing occurred in 30.5 × 24 × 21 cm conditioning chambers (Med Associates), with two aluminum side walls, a Plexiglas door and ceiling, and a white vinyl

back wall. Chambers were contained within a larger, sound-attenuating chamber. An overhead white light illuminated the chamber continuously throughout the procedures. The conditioning chamber contained a straight stainless-steel rod floor (36 rods, spaced 8mm from center to center), was cleaned with a 70% EtOH solution between uses and was scented with 1% acetic acid solution in the waste tray below the floor. Mice in the optogenetic experiment were attached to patch cables in all sessions, regardless of if light was being presented on that test day. Contextual fear conditioning consisted of three 2-sec 0.75mA scrambled foot shocks delivered through the rod floor 160, 240, and 310 seconds after mice were placed in the chamber. Mice were removed 30s after the final foot shock and returned to their home cage.

All behavioral sessions were video recorded at 30 frames/s using a near-infrared camera mounted to the interior door of the chamber. Freezing was defined as the absence of movement, except for those related to breathing. Videos were hand scored by the experimenter using Stopwatch+.

3.2.6 Retrieval and Extinction tests

On the day following context fear conditioning, mice were placed back into the conditioning context to test for fear retrieval. For optogenetic inhibition, light was delivered across a 9-minute session in 3-minute increments, from minutes 1-3 and 6-9. For this test these are referred to as ‘light on’ or ‘light off’ bins. For chemogenetic inhibition, mice were given i.p. injections of CNO 30 minutes prior to being placed in the context for a 5-minute test.

For 9 subsequent days, mice were placed back into the conditioning context without any inhibitory manipulation to extinguish fear responses. These were 9-minute sessions for the optogenetic experiment and 5-minute sessions for the chemogenetic experiment.

On the 11th day, mice again were placed in the conditioning context with inhibition to test retrieval of extinction. For optogenetics, this followed the same 9-minute light on – off – on pattern as before. For the chemogenetic experiment, mice were again injected with CNO 30 minutes prior to a 5-minute test.

3.2.7 Spontaneous Recovery

28 days after the last extinction retrieval test, mice were given an i.p injection of CNO 30 minutes before being placed in the conditioning context for 5 minutes to test for spontaneous recovery of fear.

3.2.8 Statistical Analysis

Data were analyzed using two-sided t-tests or ANOVA, using repeated measures when appropriate. ANOVAs were followed by post hoc Holm-Sidak's test for multiple comparisons. Data analysis was performed on Prism (GraphPad Software) or JMP (SAS Institute). The α value was set at 0.05 for all analyses. All data are presented as mean ±SEM.

3.3 RESULTS:

3.3.1 Optogenetic Inhibition of Ventral Hippocampal Projections to Infralimbic Cortex Did Not Affect Fear or Extinction Recall

The results in Chapter 2 show that extinction recall is associated with more c-Fos activity in vHP neurons projecting to IL than BLA. In this experiment we aimed to test the necessity of this activity for extinction behavior. Specifically, we tested whether inhibiting the vHP projection to the IL after extinction would disrupt extinction recall and potentially produce higher freezing. We used an intersectional viral approach (Fig. 1), injecting a

retrograde AAV encoding Cre-recombinase in the IL and an AAV encoding Cre-dependent ArchT and/or tdTomato in the vHP, allowing for selective recombination and expression in neurons projecting from vHP to IL. Optic fibers were implanted bilaterally above the vHP to inhibit cell bodies. Mice were given three weeks to recover from surgery, then one week of handling to habituate to being tethered to optogenetic patch cables.

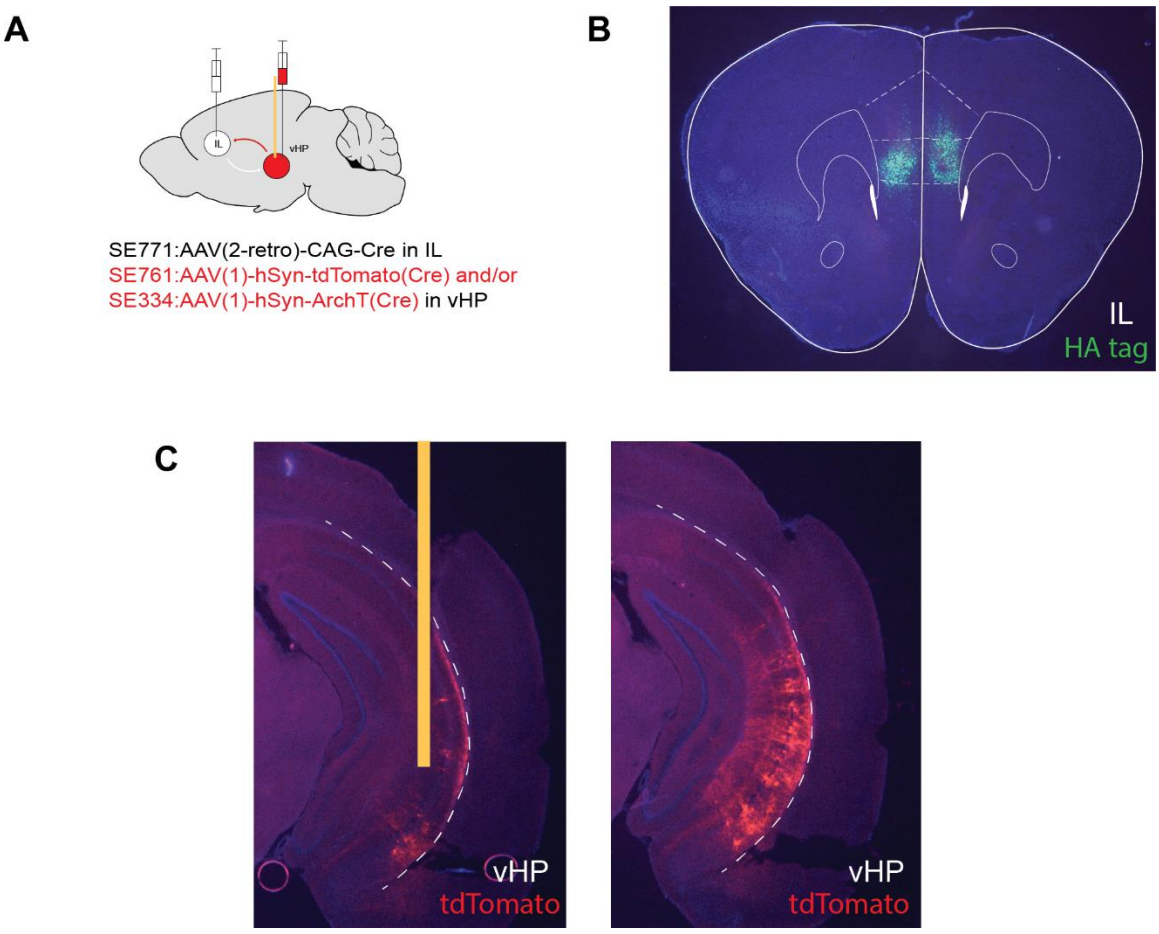

Figure 3.1: Optogenetic inhibition of vHP projections to IL.

(A) Viral strategy. (B) Retrograde-Cre injection location in the IL. (C) Optic fiber placement and viral expression in the vHP.

Mice were first context fear conditioned with no inhibitory manipulation (Fig. 2A). On the second day mice were placed in the context for fear retrieval over 9 minutes, which consisted of 3 segments lasting 3 minutes each. Upon entry to the chamber a 532nm yellow laser was delivered for 3 minutes, no light was presented for minutes 4-6, and then the light was again delivered in minutes 7-9. Light was presented at the very start of the session in order to block initial memory recall, as some previous evidence indicates that once normal recall occurs in light-off time bins, inhibition has less effect on behavior (Lacagnina et al., 2019). We hypothesized that IL inhibition would impair fear extinction and did not expect any group differences during this fear recall test. Indeed, we did not see any group differences in total time freezing or across light-on and -off time bins (Fig. 2C; RM ANOVA, no interaction, $F(2,28) = 0.237$, $p = 0.790$).

From day 3-10 mice were exposed to the fear context for 9 minutes each day without any optogenetic inhibition. Freezing behavior did decrease across extinction days, with no differences between groups (Fig. 2B; RM ANOVA, Day effect, $F(9,126) = 12.79$, $p < 0.001$; no Group effect, $F(1,14) = 0.107$, $p = 0.748$), although it is worth noting that freezing levels were low (around 20%) to begin with. Mice were tested for extinction recall on the 11th day with optogenetic inhibition as before. No differences were seen between ArchT and control groups in the percent of time spent freezing or across light-on and -off bins (Fig. 2C; RM ANOVA, no interaction, $F(2,28) = 0.508$, $p = 0.607$).

We did not observe any deficit in extinction recall, as we had hypothesized. This could be due to many factors. Both groups increased freezing on the extinction retrieval compared to the day before (Sidak's multiple comparisons, main row effect, Day10 vs. Day11, $p = 0.005$), which may suggest that something about the process of light delivery renewed some level of fear.

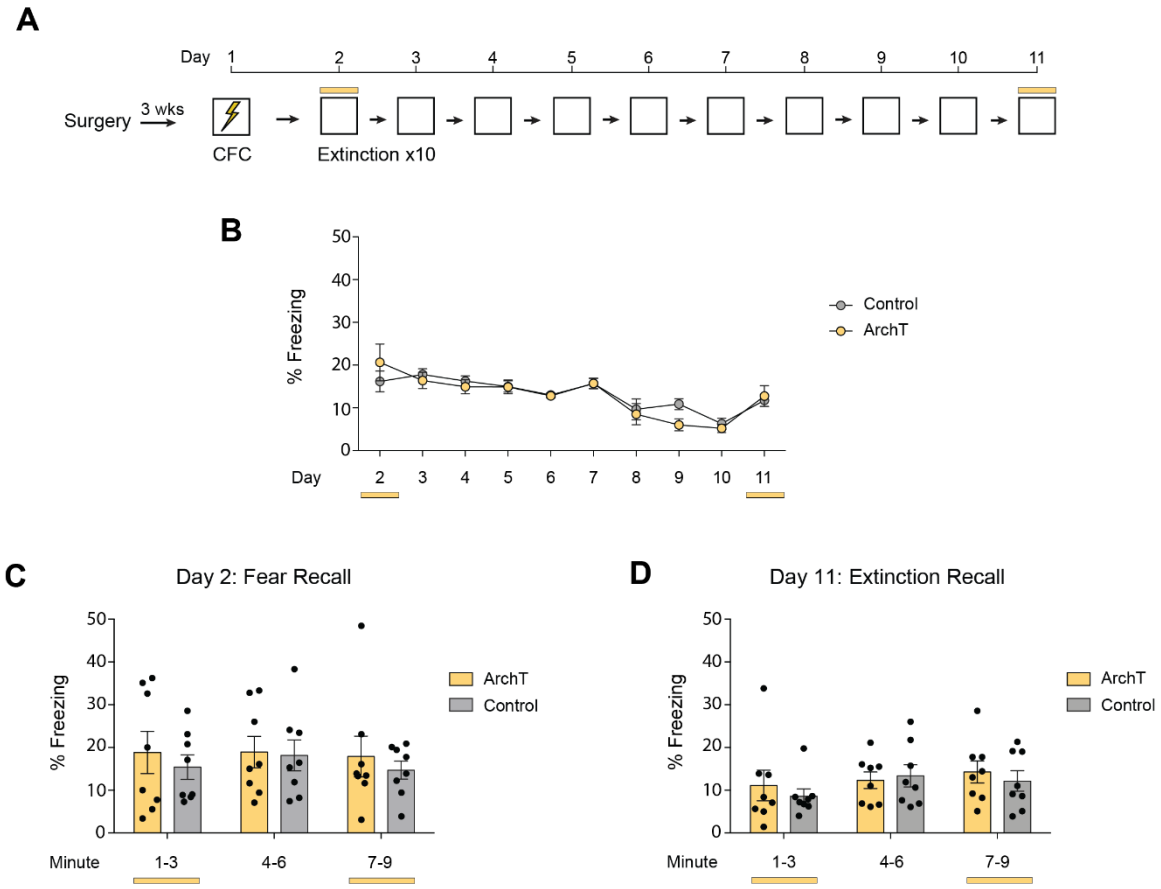

Figure 3.2: Optogenetic inhibition of vHP projections to IL did not impair extinction recall.

(A) Behavioral timeline. (B) Freezing decreased across extinction, with no differences between groups. (C) Fear recall did not show an effect of inhibition. (D) Extinction recall did not show an effect of inhibition.

Another reason we did not see behavioral differences could be that our inhibition was not effective enough. The vHP sends a substantial projection to the IL, but we are still targeting and manipulating a subset of neurons. The vHP is a large structure, and the placement of the optic fiber may result in only a portion of those neurons close to the fiber tip receiving light-induced inhibition. Figure 1C shows an example of two coronal brain sections taken 200um apart. Tracks of the optic fiber were clearly present in the first

section, but very few tdtomato labeled neurons. In the next section there is an abundance of labeled neurons containing ArchT, but depending on how far the light travels through brain tissue, it is possible that not all neurons received full optogenetic inhibition.

3.3.2 Chemogenetic Inhibition of Ventral Hippocampal Projections to Basolateral Amygdala Did Not Affect Fear or Extinction Recall

For the next experiment we used chemogenetic inhibition to examine the role of the vHP projection to the BLA. Based on our findings in Chapter 2 that fear recall was associated with more c-Fos activation of BLA than IL projecting neurons, we hypothesized that inhibiting this projection should impair fear recall. We again employed an intersectional virus strategy to specifically target vHP neurons projecting to the BLA, injecting the retrograde AAV encoding Cre-recombinase into the BLA, and an AAV encoding a Cre-dependent inhibitory chemogenic receptor hM4D(Gi) into the vHP or Cre-dependent fluorophore as a control (Fig. 3). We utilized chemogenetics in an attempt to manipulate a larger group of vHP neurons, since the previous experiment raised some concerns about optic fiber placement not allowing light to travel through the entire vHP. hM4D(Gi) receptors are exclusively activated by clozapine N-oxide (CNO), which can be delivered through intraperitoneal (i.p.) injection prior to experiments to activate the receptors and induce inhibition. As this method utilizes i.p. injections, rather than intracranial delivery, CNO should reach and inhibit all neurons expressing hM4D(Gi), so our concerns about optogenetic inhibition not fully reaching all ArchT expressing neurons do not apply.

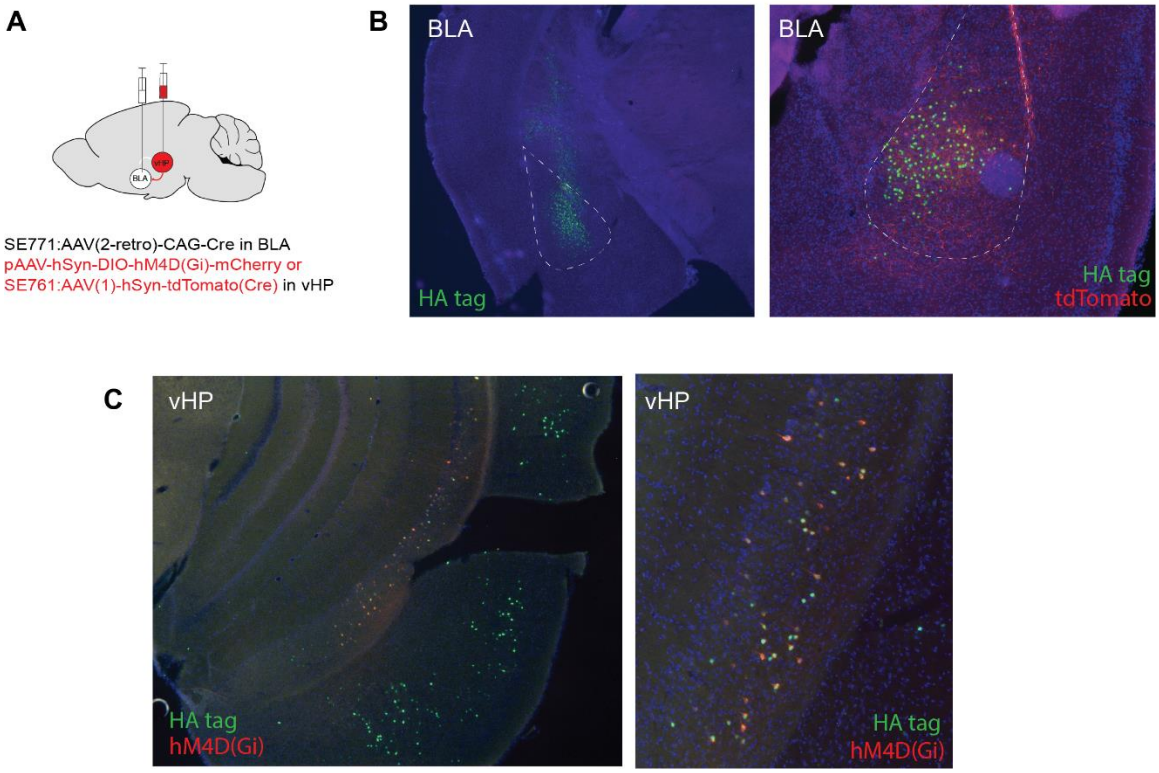

Figure 3.3: Chemogenetic inhibition of vHP projections to BLA.

(A) Viral strategy. (B) Retrograde-Cre injection location in the IL. (C) Viral expression in the vHP. hM4D(Gi) expression is represented in red, whereas green labeled neurons reflect IHC for HA to show expression of retrogradely transported Cre.

Mice were context fear conditioned on the first day with no additional manipulation (Fig. 4A). On day 2, mice were given an i.p. injection of CNO 30 minutes prior to being returned to the fear context for a 5-min retrieval test. No difference was seen between the hM4D(Gi) and control groups in the percent of time mice spent freezing to the context (Fig. 4C; t test, $t(13) = 0.412$, $p = 0.687$). Next mice were put through context extinction from days 3-10, consisting of 5-min presentations to the context each day. Mice showed a

decrease in freezing across days (Fig. 4B; RM ANOVA, Day effect, $F(10, 130) = 9.993$, $p < 0.001$; no Group effect, $F(1,13) = 0.897$, $p = 0.361$). We tested extinction recall with chemogenetic inhibition on the 11^{th} day and observed no difference between groups (Fig. 4D; t test, $t(13) = 0.799$, $p = 0.438$). Additionally, we tested for spontaneous recovery 28 days later with CNO inhibition, and also did not see any effect between groups on freezing (Fig. 4E; t test, $t(13) = 0.172$, $p = 0.866$). However, mice did not increase freezing on this test compared to the final day of extinction, showing no spontaneous recovery of fear (Sidak's multiple comparisons, main row effect, Day10 vs. SponRec, $p = 0.999$).

Overall, we saw no effects of hM4D(Gi) inhibition in this experiment. We hypothesized that inhibiting the vHP projection to BLA would decrease freezing during fear recall, which did not occur. Given that this effect has been demonstrated before under different conditions, the lack of any change in freezing calls into question the efficacy of our manipulations.

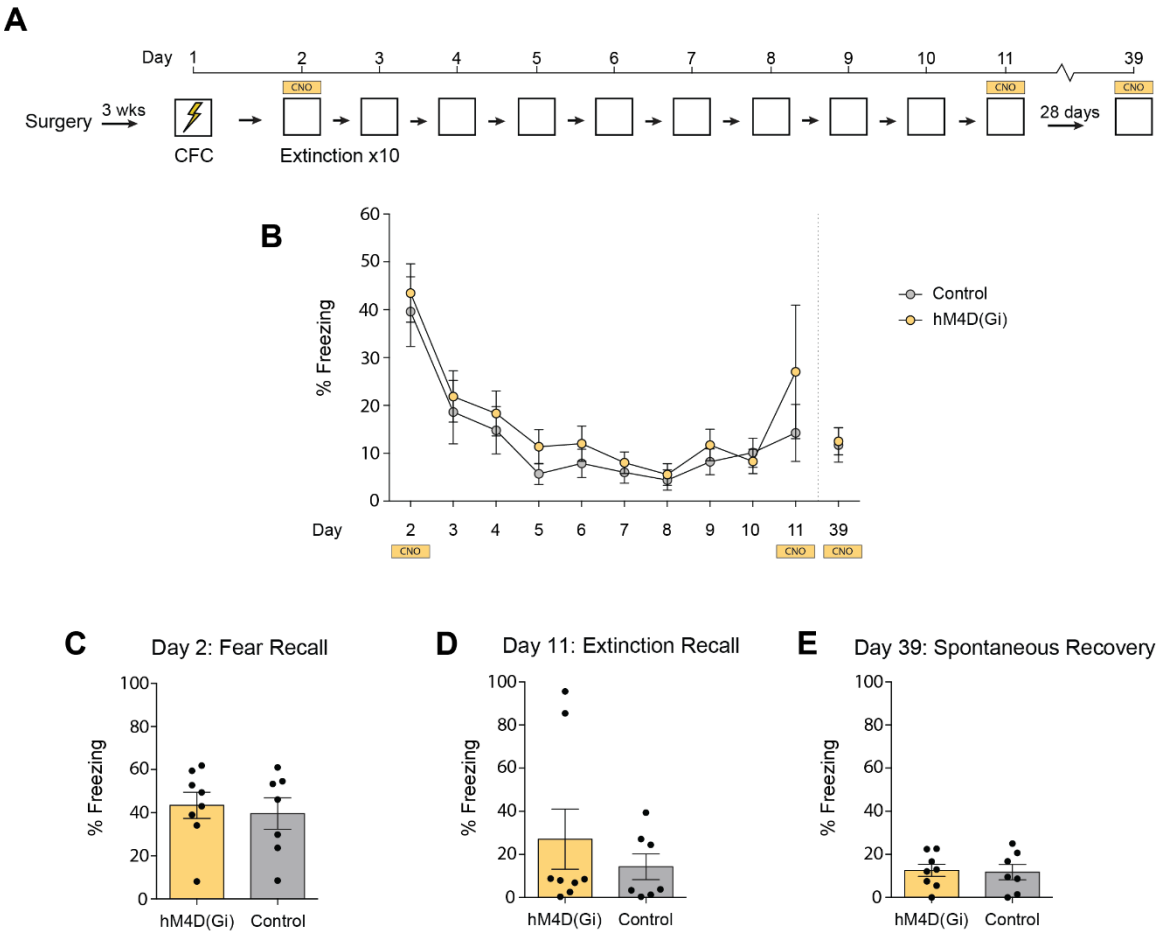

Figure 3.4: Chemogenetic inhibition of vHP projections to BLA did not impair fear recall.

(A) Behavioral design. (B) Freezing decreased over extinction. (C) No difference was seen between hM4D(Gi) groups in fear recall. (D) No difference was seen between hM4D(Gi) groups in extinction recall. (E) No effect of spontaneous recovery of fear was observed, and there was no difference between groups.

3.4 Discussion

In these experiments we used cre-dependent optogenetic and chemogenetic strategies to selectively inhibit vHP projections to IL and BLA during fear and extinction

retrieval to directly investigate their causal role in recall. In Chapter 2 we demonstrated that c-fos activity within vHP changes between fear and extinction recall, where fear activates more BLA projections than IL projections, and extinction causes the opposite and actives more IL projections than BLA projections. This pattern fits with the known roles of the BLA in fear learning and expression and the IL's involvement in extinction. However, this activity is correlational and direct manipulation of these specific projections during behavior is needed to better understand the causal impacts of this activity.

Although we were able to selectively target specific projections from vHP to BLA, we saw no behavioral differences between hM4D(Gi) and control groups. Inhibition of the vHP to BLA pathway has been shown in multiple prior experiments to impair fear recall (Xu et al., 2016; Jimenez et al., 2018). Because this effect has been previously demonstrated, we expected the BLA inhibition to be able to replicate prior findings and see a decrease in behavior. The lack of an effect in this experiment could have been due to methodological factors. Histological analysis of these animals revealed a sparce amount of neurons expressed hM4D(Gi) (Fig. 3C). Immunohistology to identify neurons expressing an HA-tag, a marker for the retrograde Cre virus, showed that the fluorophore for hM4D(Gi) was contained in almost all neurons expressing HA, the exception being a few of the most ventral neurons. As overlap of the HA tag and mCherry expressing neurons was nearly ubiquitous, we took this to mean that the injection of the hM4D(Gi) virus was enough to spread through the vHP, although we did not quantify this effect. Regardless of the recombination of the two viruses, it is possible that the sparce labeling of neurons in the vHP reflects that we were only able to inhibit a portion of BLA projecting neurons. Fear behavior was higher in this experiment than in the ArchT experiment, so a decrease in fear behavior due to inhibition should not have been masked by a floor effect of behavior. As inhibition of the vHP projection to BLA has been shown to impair fear recall (Xu et al.,

2016; Jimenez et al., 2018), the lack of results in this study suggest that methodologically it did not fully inhibit this projection.

The vHP projection to the IL has a more disputed role in extinction recall. Previous research has demonstrated that vHP projections can have both an excitatory and feed-forward inhibitory influence on the IL (Peters et al., 2010; Marek et al., 2018). We cannot tell from this experiment what influence our ArchT inhibition had in the IL, if any. Our histology showed that although there was substantial labeling of vHP neurons with tdTomato, the optic fiber placement in some animals was not directly over the bulk of the labeled neurons. This could mean that not all viral infected cells were inhibited by opsin activation of ArchT receptors. Additionally, inhibiting extinction recall, effectively removing suppression of fear, might have had a relatively small effect on freezing behavior. Mice in this experiment had low freezing to begin with and both groups showed an increase of fear on the extinction retrieval test, suggesting some level of renewal of fear. Our setup included a cover over the patch cable connection, so visible light was minimal, but it cannot be ruled out that light, heat, or experimenter cues may have overall influenced behavior on this day. As freezing levels were only around 20% even on the fear retrieval day, it is possible that any small influence to increase fear behavior would be masked by the overall increase in freezing or reach a ceiling effect. Impaired extinction recall would not be likely to drive freezing any higher than their behavior on the day of fear recall, just remove the suppression of that fear.

It could also be the case that our inhibition of vHP to IL did work, and that inhibiting this pathway has no effect on freezing behavior in our paradigm. This would suggest that the vHP input to IL is not necessary for context extinction recall. Work assessing this pathway has primarily looked at contextual gating of cued fear, such as Marek et al.'s study showing that inhibition of this pathway diminished cued fear renewal. Extinction of cued

fear is highly context-specific, and contextual information is influential as to whether fear should be suppressed or renewed in a given situation. It is possible that after context extinction, ventral hippocampal contributions to the IL are not needed to disambiguate the association between the context and fear. Memory for extinction has been localized to particular sets of neurons in the amygdala (Herry et al., 2008), so it could be that context extinction memory can be recalled without activity in the vHP projection to the IL, but instead through other pathways. But how does this result fit with our data in Chapter 2? We found that during extinction recall there is more c-Fos activity in vHP projections to IL than BLA. Our results in this chapter, if reliable, would suggest that although the vHP is conveying contextual information to the IL during extinction retrieval, this activity may not be necessary to recall the extinction memory and suppress fear. Future work should continue to investigate the role of vHP projections. Direct manipulation of these pathways will lead to a better understanding of the role vHP activity has in influencing fear and extinction recall and activity in the IL and BLA.

Chapter 4: Stimulation of SST interneurons in vHP impairs fear recall and extinction learning

4.1 INTRODUCTION:

The hippocampus is required for contextual fear learning and maintains distinct representations of fear and extinction memory (Fanselow, 2000; Lacagnina et al., 2019). Projections from the ventral hippocampus (vHP) to the amygdala and prefrontal cortex are thought to be responsible for conveying contextual dependency of cued fear and extinction memory, and our previous work suggests a role for vHP projections in mediating contextual fear and extinction recall. Yet there is still much to understand about how the vHP might influence contextual fear extinction. In this study we sought to assess the necessity of the vHP and prefrontal cortex (PFC) during context fear retrieval and extinction learning and retrieval.

Much of what we know about vHP involvement in contextual fear learning derives from lesion studies. These studies demonstrate a role for vHP in behavioral inhibition and modulation of anxiety, rather than context learning *per se*. vHP lesions do not impair contextual fear conditioning or spatial navigation in a water maze, but do have anxiolytic effects in tests of anxiety like the elevated plus maze, hyponeophagia, and light/dark box (Kjelstrup et al., 2002; Bannerman et al., 2003; McHugh et al., 2004; McDonald et al., 2006). Lesions to vHP also cause deficits to inhibiting behavior when contextually relevant. When rats are trained to discriminate two arms of a Y maze, one of which is reinforced, they will learn to reverse this association better in a different context, an effect that is thought to be due to a context-specific inhibitory association to the non-reinforced arm. vHP lesioned rats learn the discrimination task similarly to controls, but show enhanced reversal learning in the original context, displaying less inhibition to approach the non-reinforced arm (McDonald et al., 2001, 2006). Additionally, hippocampal

knockout of NMDA receptors causes mice to fail to inhibit their approach to incorrect beacons in a water maze, but only when the starting location is closer to the inappropriate beacon, an effect attributed to the vHP (Bannerman et al., 2014). These studies show a role for vHP in modulating innate effects of anxiety and behavioral inhibition.

vHP lesions do influence contextual fear, as vHP lesioned rats show less shock-reactivity to un-signaled shocks and reduced fear expression during retrieval tasks (Bast et al., 2001; Maren and Holt, 2004; McDonald et al., 2018). Silencing vHP adult born neurons also impairs acquisition and recall of contextual fear memory (Huckleberry et al., 2018). However, less work has been done to understand the ventral hippocampal role in the extinction of context fear. Extinction, the gradual suppression of a conditioned response in the absence of an unconditioned stimulus, could be considered as a form of behavioral inhibition. As such, the vHP may play a role in suppressing fear after learning though extinction that the context no longer predicts shock delivery. The vHP may not just be an output region for contextual memory involved in contextual learning, but potentially positioned in a way to influence extinction learning through inhibiting inappropriate behavior and regulating emotional responses.

The PFC is another region implicated in fear expression and extinction. Inactivation of the prelimbic (PL) region impairs fear expression, whereas inactivation of the infralimbic (IL) region impairs extinction acquisition and memory (Sierra-Mercado et al., 2011; Sotres-Bayon et al., 2012). Projections from the IL to the basolateral amygdala facilitate extinction of cued fear conditioning (Bukalo et al., 2015). The vHP sends projections to both PL and IL (Hoover and Vertes, 2007). The projections from the vHP to the prefrontal cortex have also been implicated in extinction processing, although the influence of activity along this pathway is disputed. As discussed in Chapter 3, vHP projections to IL can be both excitatory and net inhibitory through feed forward inhibition

(Peters et al., 2010; Rosas-Vidal et al., 2014, 2018; Marek et al., 2018). The opposing roles of the PL and IL, and potentially bidirectional influences of vHP projections in this region, suggest the PFC plays an executive role in modifying behavior in ambiguous or uncertain conditions and likely uses contextual input from the hippocampus to do so. However, there is a lack of studies that have investigated the influence of the prefrontal cortex on contextual fear conditioning and extinction rather than cued associations guided by context.

Here we use a dual virus intersectional approach to optogenetically stimulate somatostatin (SST) positive interneurons. These GABAergic interneurons are suggested to influence the strength and specificity of learning through modulation of pyramidal neuron firing. While primarily an inhibitory force on pyramidal neurons, these interneurons regulate oscillation patterns through specific timed activity. Stimulating SST interneurons should increase inhibition of the surrounding pyramidal neurons or cause dysregulation of normal functioning oscillatory activity. SST interneurons in the PFC have also been demonstrated to exhibit properties of memory storage, such as learning dependent potentiation of synaptic transmission and cue-specific activation during memory retrieval (Cummings and Clem, 2020). SST interneurons have wide connections to pyramidal neurons in the vHP, which additionally provides a way to influence a large portion of the hippocampus through a smaller targeted viral injection. Optogenetic stimulation of SST interneurons therefore presents an appealing method to inhibit excitatory neural ensembles and assess a circuit mechanism poised to modulate hippocampal and prefrontal memory processing.

In these experiments we context fear conditioned animals, then used optogenetic stimulation of SST interneurons during retrieval and extinction sessions to assess the role of the ventral hippocampus and prefrontal cortex during context fear recall and extinction. We hypothesized that SST activation would lead to regional inhibition, and thus potentially

disrupt memory retrieval. We show that SST stimulation in the ventral hippocampus during fear retrieval reduced freezing to the conditioning context and impaired extinction learning. SST stimulation in the PFC did not show any effects on context fear or extinction retrieval; however, we were able to demonstrate an inhibition of extinction learning in a cued fear conditioning paradigm. These results show a unique role for the ventral hippocampus in contextual fear recall and extinction learning and highlight potential differences in cued and contextual learning in fear circuitry.

4.2 METHODS

4.2.1 Animals

Adult male and female C57BL6/J mice (Experiment 1: n=16 (8M, 8F); Experiment 2: n=20 (10M, 10F); Experiment 3: n=20 (10M, 10F)) mice approximately 2-4 months of age were used for all experiments. Mice were housed with littermates in groups of 3-4 in plastic cages with woodchip and paper crinkle bedding and maintained on a 12hr light/dark cycle (7:00-19:00 light on) in a temperature- and humidity-controlled vivarium. Food and water were provided *ad libitum*. Experiments were conducted during the light phase. Mice were randomly assigned to groups by cage before the start of each experiment. As sex by genotype statistical interactions were not present, male and female data were aggregated. All procedures were approved by the University of Texas at Austin Institutional Animal Care and Use Committee.

4.2.2 Viruses

Adeno-Associated Viruses (AAVs) SE928:AAV(7)-SST-Cre, BZ118:AAV(7)-h56D-(EGFP)^{Cre} and MM104:AAV(1)-h56D-(ChR2-EGFP)^{Cre} were made at UT Austin in the lab of Boris Zemelman.

4.2.3 Surgery

2% isoflurane (1.0 L/min) vaporized in pure oxygen was used to induce anesthesia, followed by 1.5% isoflurane (0.75 L/min) to maintain stable anesthesia after mice were placed in a stereotaxic frame. A mixture of SE928:AAV(7)-SST-Cre and either BZ118:AAV(7)-h56D-(EGFP)^{Cre} or MM104:AAV(1)-h56D-(ChR2-EGFP)^{Cre} was infused bilaterally using a NanoJect II microinjector (Drummond) targeting the vHP (50nl/injection, M/L +/-3.5, A/P -3.2, D/V -3.4 and -3.8) or PFC (75nl/injection, M/L +/-0.3, A/P +1.8, D/V -2.9). Optogenetic fibers (0.22 NA, 200µm core) were implanted bilaterally 200µm above the injection sites, using the same coordinates for vHP (3.5mm fiber, D/V -3.5) and approaching at an angle for IL (3.0mm fiber, 25-degree angle, M/L +/-1.65, A/P +1.8, D/V, 2.92). The fibers were secured in place with a layer of dental acrylic. Mice were injected subcutaneously with carprofen (10mg/kg), and buprenorphine XR (3.25mg/kg) in sterile saline (0.9%) to provide analgesia. Subjects were given ~21 days to recover and allow for viral expression.

4.2.4 Optogenetics:

Cranial implants were bilaterally connected via fiber optic patch cables to a light source interfaced with a FC/PC rotary joint (Doric Lenses). ChR2 stimulation was delivered with a 17.2 mW, 470 nm LED (ThorLabs) delivered in 15ms pulses at 20 Hz with an intensity of 1.1 mW at the end of the fiber optic implant.

4.2.5 Context fear conditioning:

Prior to testing mice were handled to habituate to the experimenter and the process of being tethered to optogenetic patch cables. Mice were gently handled for 2 min per day for the first two days. The third day mice were put in a gentle restraint and fibers were cleaned. On days 4-6 mice were gently restrained, attached to dummy patch cables, and allowed to explore a clean cage with familiar bedding.

Behavioral testing occurred in 30.5 × 24 × 21 cm conditioning chambers (Med Associates), with two aluminum side walls, a Plexiglas door and ceiling, and a white vinyl back wall. Chambers were contained within a larger, sound-attenuating chamber. An overhead white light illuminated the chamber continuously throughout the procedures. The conditioning chamber contained a straight stainless-steel rod floor (36 rods, spaced 8mm from center to center), was cleaned with a 70% EtOH solution between uses and was scented with 1% acetic acid solution in the waste tray below the floor. Mice were attached to optogenetic patch cables in all sessions, regardless of if light was being presented on that test day. Contextual fear conditioning consisted of three 2-sec 0.75mA scrambled foot shocks delivered through the rod floor 160, 240, and 310 seconds after mice were placed in the chamber. Mice were removed 30s after the final foot shock and returned to their home cage.

All behavioral sessions were video recorded at 30 frames/s using a near-infrared camera mounted to the interior door of the chamber. Freezing was defined as the absence of movement, except for those related to breathing. Videos were hand scored by the experimenter using Stopwatch+.

4.2.6 Retrieval and Extinction tests

The day following context fear conditioning mice were placed back into the conditioning context for 12 minutes. Optogenetic stimulation was delivered in 3-minute increments, from minutes 1-3 and 6-9. For this test these are referred to as ‘light on’ or ‘light off’ bins.

On subsequent days, mice were placed back into the conditioning context for 6 minutes, either with or without optogenetic stimulation, to test for further retrieval and extinction.

4.2.7 Auditory fear conditioning and extinction

Auditory fear conditioning occurred in the same fear conditioning apparatus, with a tiered grid flooring and 1% vanilla extract scent. Conditioning consisted of a 3-minute baseline period followed by 4 pairings of a 30sec, 80 dB, 5000 Hz tone culminating in a 2sec 0.75 mA shock. Tone-shock pairings were separated by 30-60 seconds, and mice were removed from the chamber 30 seconds following the last shock. Extinction occurred in a third context with wood chip bedding on the floor, a curved back wall insert, and 2% lemon soap scent. Extinction consisted of 20 30sec tones with 20sec intertrial intervals. On the following day, retrieval was assessed in the extinction context and consisted of 4 tone presentations.

4.2.8 Immunohistochemistry

Mice underwent optogenetic stimulation for 6 minutes in the fear conditioning context, similar to retrieval sessions, then ninety minutes later were deeply anesthetized with ketamine/xylazine (150/15 mg/kg) and transcardially perfused with 1× phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA) in 1× PBS. Brains were extracted and post-fixed overnight at 4°C in 4% PFA and then transferred to a 30% sucrose

in 1× PBS at 4°C for two days. 35 μm coronal sections were collected on a cryostat. For immunohistochemistry, sections were washed in 1× PBS and blocked at room temperature (RT) for 1.5 h in 5% normal donkey serum (NDS) in 1× PBS with 0.5% Triton-X (PBS-T). Sections were incubated with primary antibodies (1:2,000 rabbit anti-c-Fos polyclonal antibody, Synaptic Systems #226-003) diluted in 5% NDS in 1× PBS-T overnight at room temperature. Sections were rinsed in 1× PBS-T and incubated in secondary antibodies (Alexa Fluor 555-conjugated AffiniPure Donkey Anti-rabbit IgG, Jackson ImmunoResearch Laboratories) in 1× PBS-T for 2 h at RT. Sections were washed in 1× PBS, mounted onto slides, and stained with 1:1000 DAPI for 5 minutes before being coverslipped with Fluoromount-G (SouthernBiotech).

4.2.9 Imaging and quantification

4-6 images per animal were taken of the target region on a Zeiss ZoomScope and Zen imaging software. Fluorescent channels were separated in ImageJ to manually count neurons, using ROI sets to compare between channels. A cell was considered positive for viral labeling if a dense outline of the soma was visibly distinct from background labeling and labeled fibers. A cell was considered positive for c-Fos labeling if fluorescence level in the soma was 2x background levels of fluorescence. Image counts were averaged for each subject and are displayed as mean +/- SEM.

4.2.10 Statistical Analysis

Data were analyzed using two-sided t-tests or ANOVA, using repeated measures when appropriate. Significant ANOVAs were followed by post hoc Holm-Sidak’s test for multiple comparisons. Data analysis was performed on Prism (GraphPad Software) or JMP

(SAS Institute). The α value was set at 0.05 for all analyses. All data are presented as mean ±SEM.

4.3 RESULTS

4.3.1 vHP SST interneuron stimulation decreases context freezing during retrieval

ChR2 was expressed in ventral hippocampal SST interneurons using a dual viral strategy consisting of SE928:AAV(7)-SST-Cre to express Cre in SST cells, and the inhibitory targeting BZ118:AAV(7)-h56D-(EGFP)^{Cre} or MM104:AAV(1)-h56D-(ChR2-EGFP)^{Cre}. Mice underwent surgery for viral microinjection and optic fiber implantation and were given three weeks for recovery. Mice then went through context fear conditioning while attached to fiber optic patch cables without any light delivery. The next day, fear retrieval was assessed over 12 minutes, with alternating 3-minute periods of blue light delivered at 20 Hz (15ms pulses) or light off. A two-way RM ANOVA found no interaction effect of Group by 3-minute Bins ($F_{(3,42)}$ = 0.8638, p = 0.467), but did find an effect of Group ($F_{(1,14)}$ = 7.551, p = 0.0157). During the first light-on period, the ChR2 group froze significantly less to the fear context than the control group (Sidak's multiple comparison, $t_{(14)}$=2.614, p = 0.0451). Upon termination of light stimulation, freezing rose to a similar level as the control mice ($t_{(14)}$=1.426, p = 0.501). In the second light on period freezing again dropped to be lower than the control group, but was not significantly different ($t_{(14)}$=2.541, p = 0.054). When tested 24 hours later with no light stimulation, there was no difference in freezing between ChR2 and control mice ($t_{(14)}$=0.496, p = 0.627). These results demonstrate that stimulation of vHP SST interneurons decreases the expression of contextual fear during retrieval.

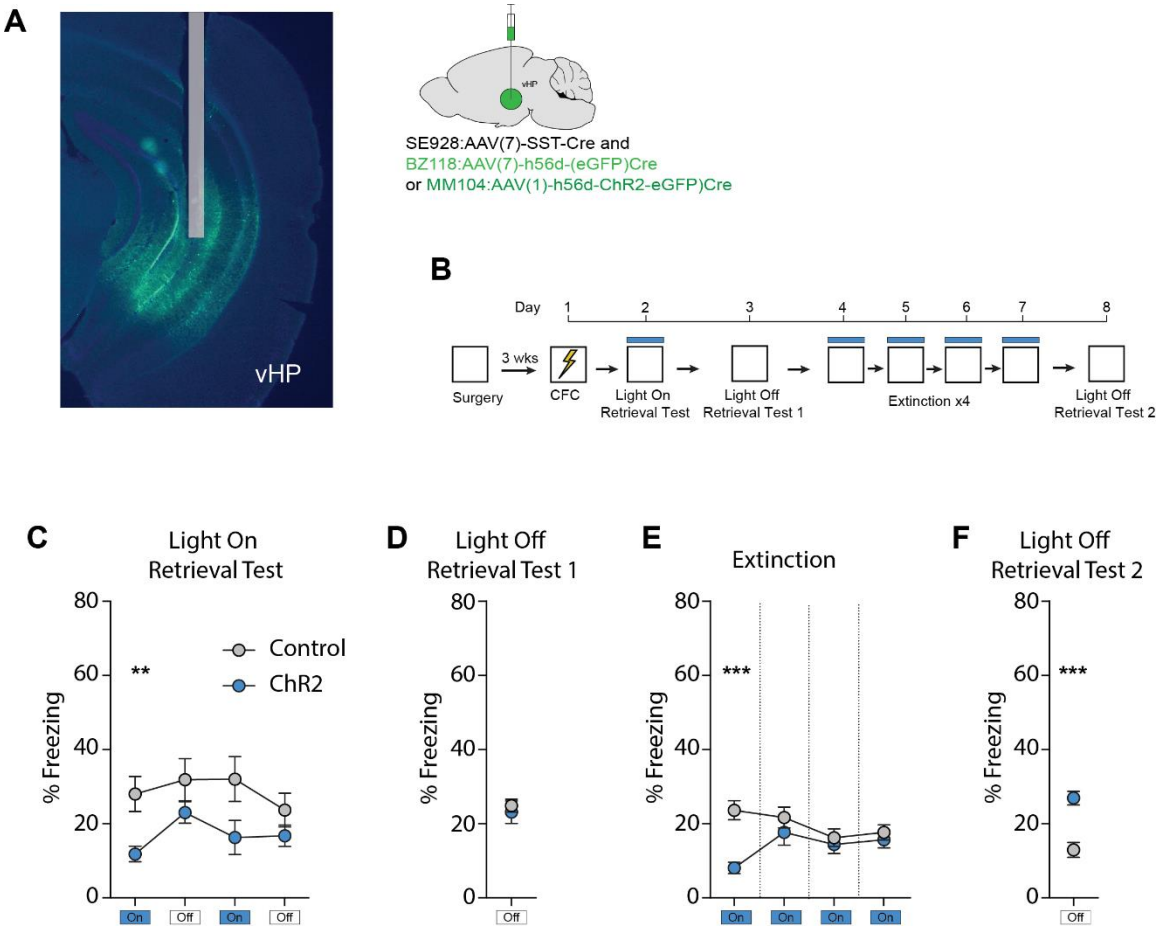

Figure 4.1: Stimulation of SST interneurons in vHP impairs fear recall and extinction

(A) Representative image of vHP showing virus and optic fiber placement as well as experimental design. (B) Timeline. (C) Freezing behavior during light-on and -off bins of fear retrieval test. Freezing decreased in the ChR2 group during the initial stimulation period. (D) Retrieval without stimulation was no different between groups. (E) During extinction, freezing initially was reduced in the ChR2 group, then showed no difference from controls. (F) Extinction retrieval was impaired, with higher freezing in the ChR2 group.

4.3.2 vHP SST interneuron stimulation during extinction impairs extinction retrieval

To test the effects of vHP SST stimulation during extinction mice underwent 4 days of context exposure with blue light presentation. There was a significant interaction effect of Group by Day ($F_{(5,70)} = 13.22$, $p < 0.001$). The ChR2 group again showed a significant decrease in freezing during the first extinction day (Sidak Multiple comparisons, $t_{(14)}=4.579$, $p < 0.001$), but there was no difference between groups on the next three days of context exposure with stimulation (Ext2, $t = 1.168$, $p = 0.8165$; Ext3, $t = 0.5436$, $p = 0.995$, Ext4, $t = 0.5855$, $p = 0.993$). When the mice were returned to the context chamber for a final retrieval test without light presented, the ChR2 mice froze significantly more than control mice ($t_{(14)}=4.108$, $p < 0.001$). This higher freezing during the extinction recall test suggests that stimulation impaired extinction learning on the previous extinction days.

4.3.3 PFC SST interneuron stimulation has no effect on context fear retrieval

SST interneurons in the prefrontal cortex were targeted in a separate group of mice. Mice underwent surgery for virus injection and optic fiber implantation. Injection coordinates were aimed at the IL, but the spread of the virus was larger than intended and did include some of the PL. Thus we will refer to this group as stimulating the PFC. Three weeks later mice received context fear conditioning. Fear retrieval was assessed over 12 minutes, with alternating 3-minute periods of blue light delivered at 20 Hz (15ms pulses) or light-off. Mice froze at similar levels during fear retrieval in both the light-on and light-off periods, and no significant differences were seen between the ChR2 and control group (Two-way RM ANOVA, no interaction effect, $F_{(3,42)} = 1.290$, $p = 0.291$; Sidak Multiple comparison light on 1, $t = 0.673$, $p = 0.939$). No group difference was seen in a light-off retrieval session the following day ($t = 1.427$, $p = 0.499$). On the next three days of

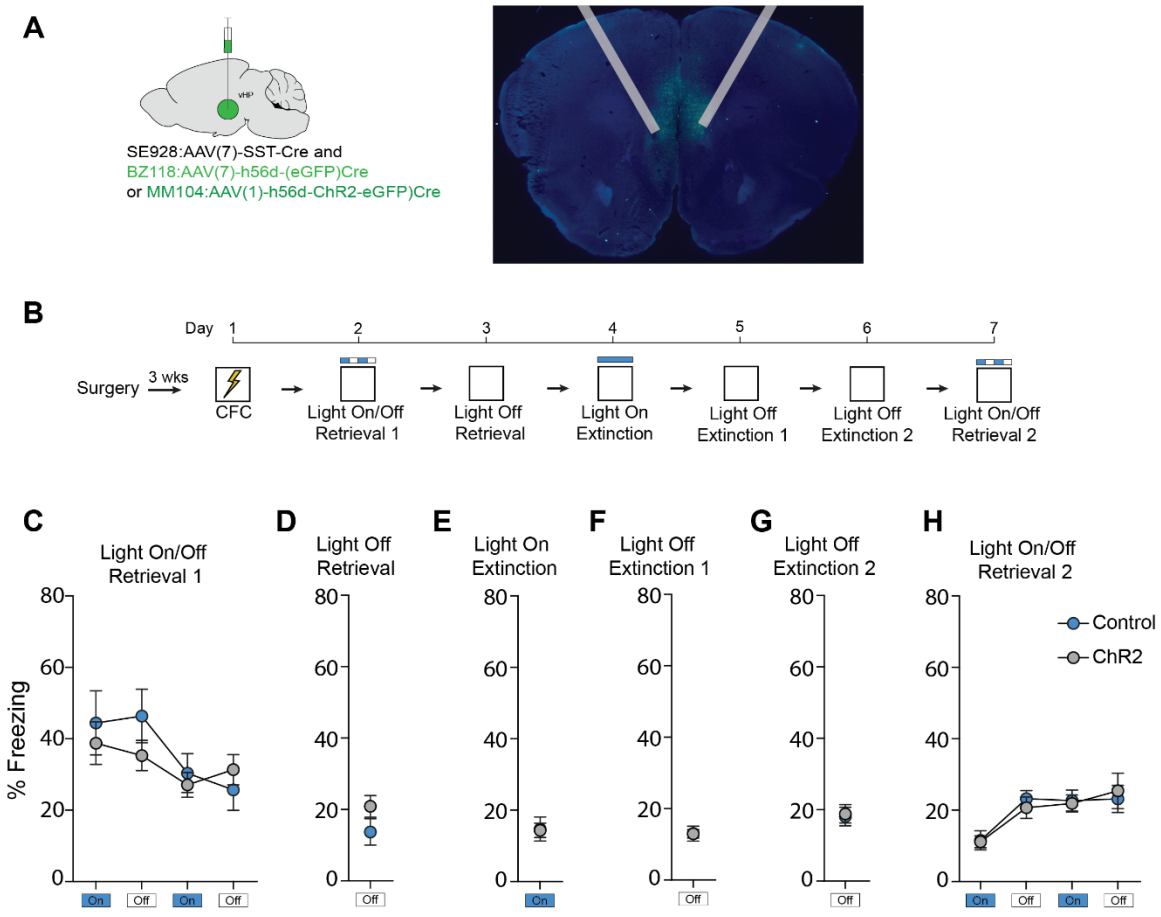

Figure 4.2: Stimulation of SST interneurons in the PFC

(A) Experimental design and representative image of virus and optic fiber placement in the PFC. (B) Timeline. (C-H) No difference between ChR2 groups was seen during context fear retrieval, across extinction, or extinction retrieval.

extinction, both ChR2 and control groups froze at similar levels both with and without light stimulation. On the last test day we again tested mice with alternating periods of light on and light off, and saw no differences between the two groups (No interaction, $F_{(3,54)}$ = 0.3148, p = 0.8146, or group effect, $F_{(1,18)}$ = 0.014, p = 0.906). These results suggest that in these animals, stimulating SST interneurons in the IL had no effects on freezing behavior during context fear retrieval or extinction.

4.3.4 PFC SST interneuron stimulation increases freezing after tone fear conditioning and extinction

Next, we sought to test if our SST stimulation, which should increase inhibition, could replicate an experiment by Do-Monte et al. that used ArchT inhibition during auditory fear extinction. Do-Monte et al. have shown that silencing glutamatergic neurons in the IL during extinction training had no effect on within-session extinction but impaired extinction retrieval on the following inhibition-free day. We thus attempted to replicate this experiment to test our optogenetic manipulation.

The same groups of mice from the previous experiment were auditory fear conditioned in a new context, consisting of a different floor grid and scent. Baseline freezing to this new context was low, suggesting little if any generalization of fear across contexts. Auditory conditioning consisted of 4 pairings of a 30-second 80-dB tone culminating in a 2-second 0.75 mA shock. Extinction occurred the following day in a third context and consisted of 20 tones without shock. Optogenetic stimulation occurred during the 30-second tones. ChR2 mice froze significantly more than control mice during the first two tones, but extinguished across the session and froze at similar levels to the control group in all other tone presentations (Fig. 4.3C: ANOVA, no effect of group: $F_{(1,18)} = 0.014$, $p = 0.908$). In a light free retrieval session the following day, ChR2 mice again froze significantly more than control mice (Fig. 4.3D: $F_{(1,54)} = 8.533$, $p < 0.01$). This is similar to the results seen by Do-Monte et al. and suggest that our optogenetic manipulations are effective in impairing auditory fear extinction, but not in our original contextual fear paradigm.

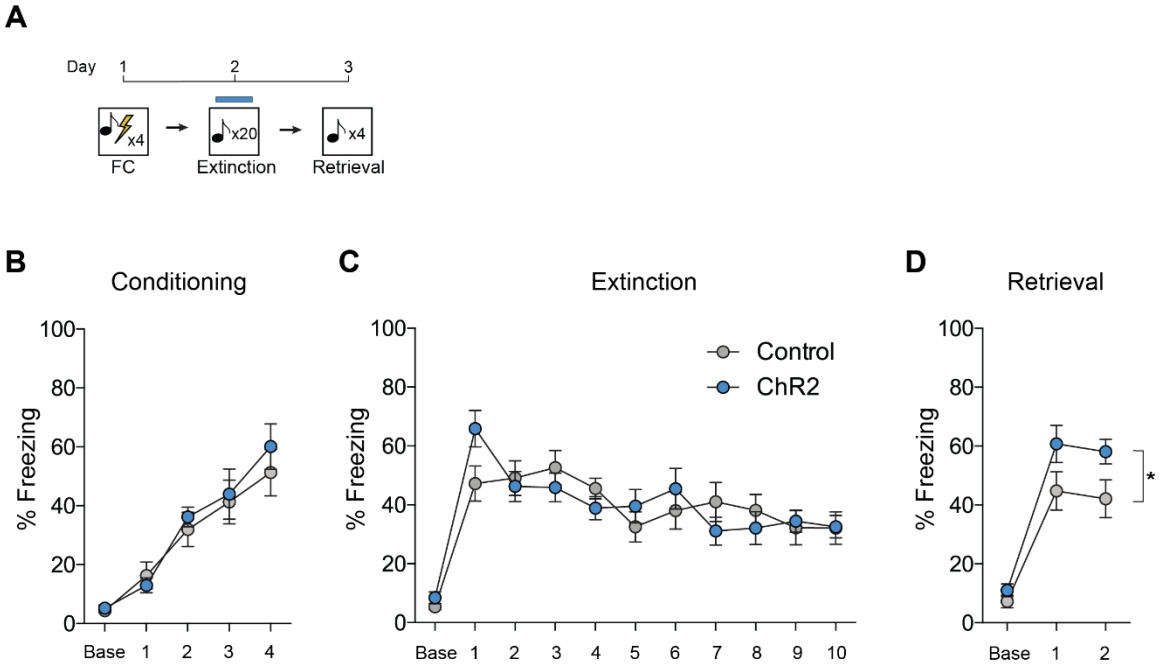

Figure 4.3: SST stimulation impairs auditory fear extinction.

(A) Timeline. (B) Tone conditioning was similar for both groups. (C) Extinction with SST stimulation. ChR2 mice initially displayed higher freezing, but extinguished similar to controls. (D) ChR2 mice froze more than controls during extinction retrieval.

4.3.5 IL specific SST interneuron stimulation has no effect on fear or extinction retrieval

A third experiment was performed to test a smaller injection in the PFC to better isolate the infralimbic cortex. We used a simplified contextual fear paradigm, where SST stimulation occurred only during extinction sessions, to compare to our vHP results and auditory cue conditioning results. Mice were context fear conditioned, then went through four days of context extinction with SST stimulation at 20 Hz (15ms pulses). Mice were then tested without any light stimulation in a subsequent retrieval test. No differences between groups were seen during extinction sessions, or during retrieval.

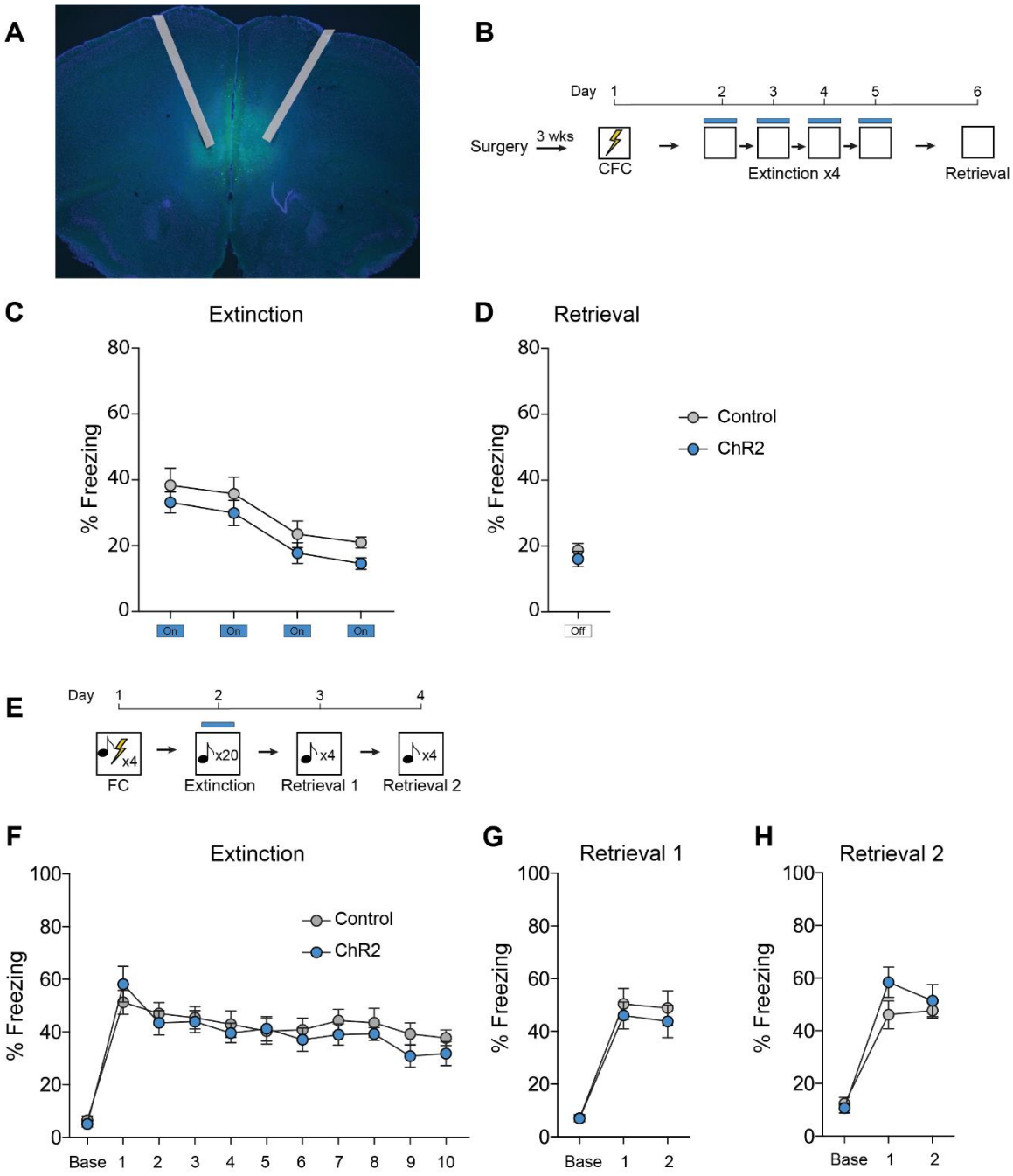

Figure 4.4: IL specific injections did not affect fear or extinction behavior.

(A) Representative image showing low expression of virus. (B-D) Timeline, stimulating during extinction learning had no effect on extinction retrieval. (E-F) Timeline, stimulating during auditory extinction learning had no effect.

To confirm the efficacy of our manipulation, these groups of mice were then auditory fear conditioned similar to the previous experiment and underwent auditory fear extinction with light presentation during the tones. No difference between groups was seen during tone extinction. The following day mice were tested in a light free retrieval trial, but no difference in freezing was seen between the groups that received SST stimulation during extinction and the control group. Histological verification of injection locations and viral spread showed that the smaller injection volume resulted in more variable virus infection, with some animals displaying minimal amounts of neurons reporting GFP in the IL and some showing good coverage in only one hemisphere. As this experiment failed to replicate the impaired freezing during auditory tone retrieval seen in our previous experiment, it is possible that this attempt at more specific IL targeting did not result in a significant stimulation of IL SST interneurons.

4.3.6 Histology confirms activation of GFP labeled cells and some colocalization with SST

After experiments, histology was performed to assess c-Fos activity within GFP labeled cells, and colocalization with SST. IHC for c-Fos confirmed an increase in the percent of GFP cells that were also positive for c-Fos in both the vHP and PFC ChR2 groups compared to controls (One-way ANOVA, $F(2,26) = 20.38$, $p < 0.001$.) As both controls showed low c-Fos expression within these populations, the control groups were combined for these analyses. IHC against an SST antibody however showed an interesting pattern. Some cells were clearly labeled for both GFP and SST, but many GFP positive cells did not show clear SST labeling within the some. Many GFP labeled cells in the vHP were located in the lacunosum moleculare, rather than the stratum oriens. There is a high

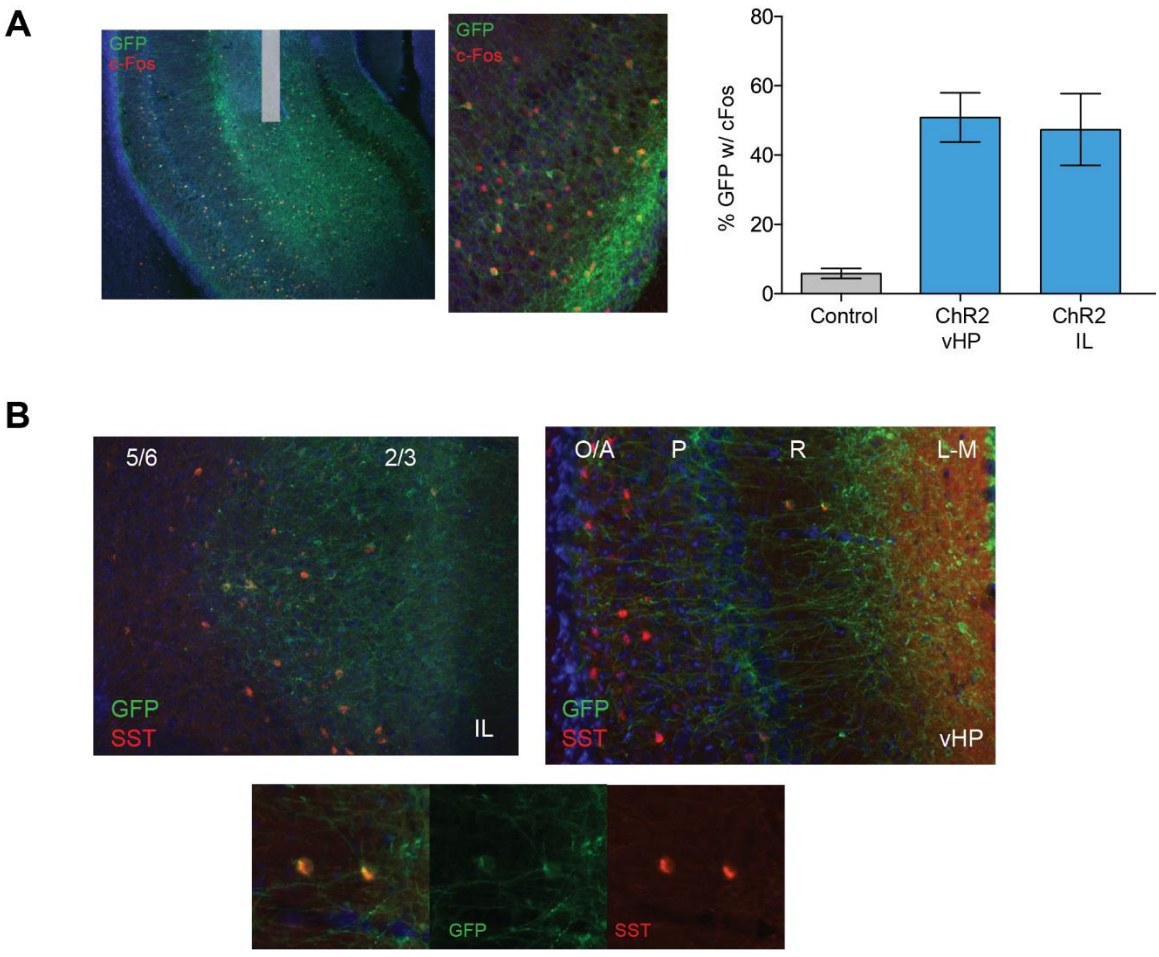

Figure 4.5: Histology for c-Fos activation and SST

(A) Representative images of c-Fos IHC. Both ChR2 groups had higher c-Fos activity within GFP labeled cells, confirming ChR2 excitation. (B) IHC for SST is not a perfect overlap, with some GFP cells also positive for SST, but many are not.

degree of non-somatic SST antibody labeling here, but it is unclear if the GFP cells are SST+ based on this histology. IHC in the IL similarly showed a low degree of somatic SST labeling within GFP+ cells. Given that there is a substantial amount of non-somatic labeling with this SST antibody, running another histological analysis such as fluorescent *in situ* hybridization may provide a more complete picture of the overlap of viral infection

and cell expression of SST. While the behavioral effects seen in this chapter suggest that our optogenetic stimulation led to general inhibition, and GFP labeled cells do not look like excitatory pyramidal neurons, it is possible that we might be targeting a larger population of interneurons than just SST.

4.4 DISCUSSION

In this series of experiments, we investigated the role of the vHP and PFC in context fear and extinction using optogenetic stimulation of SST interneurons. The vHP is recognized for its involvement in context fear learning, but less is known about its role in extinction. On the other hand, the PFC, IL in particular, is known to influence auditory cue extinction, but whether it is needed for context extinction is not understood. There is still much to learn about how these regions influence context fear and extinction. Here we stimulated SST interneurons to induce inhibition or dysregulation of the target areas to test the necessity of vHP and PFC in expressing and suppressing context fear. We demonstrate that stimulating SST interneurons in the vHP reduces context freezing during initial fear retrieval as well as impairing fear extinction learning, as seen in a stimulation free extinction retrieval test. We also show that SST stimulation in the PFC had no effect on context fear and extinction retrieval but did impair auditory fear extinction. These results clarify the roles of the vHP and PFC in fear and extinction, while also underscoring the differences between contextual and discrete cue association learning.

We focused on stimulating SST interneurons to cause widespread local inhibition of principal neurons, but these cells likely have a more nuanced role in modulating local activity. SST interneurons also can inhibit parvalbumin interneurons and lead to the disinhibition of principal neurons (Cummings and Clem, 2020). SST interneurons are a

diverse population, containing various receptor and peptide expression, and distinct ensembles of SST interneurons have been shown to encode fear and reward memory (Cummings et al., 2022). Our method of targeting SST interneurons resulted in GFP positive cells across the layers of CA1, including in the radiatum and lacunosum moleculare, rather than being confined to the stratum oriens where the impact of SST interneurons has been most frequently studied. SST cells across hippocampal layers have differing synaptic connections and may be differentially contributing to local circuit activity. Our results suggest that our SST interneuron stimulation is having a net inhibitory effect, as our cued fear extinction findings in the second experiment replicate a previous effect seen by inhibiting principal neurons with ArchT. However, given SST interneurons ability to finely modulate local circuitry, stimulation could have an effect through not necessarily decreasing pyramidal cell activity, but by disrupting synchronized oscillation activity or other subtle modulation of the local circuit.

Our results show support that the vHP is involved broadly in contextual fear retrieval and extinction learning. We show that freezing behavior decreases with SST stimulation during fear retrieval, which is indicative of impaired fear memory retrieval. This decrease in freezing might reflect impairment in signaling to the basolateral amygdala, an integral region for fear association. On the other hand, SST stimulation during extinction sessions led to an increase of freezing behavior on the final, no-stimulation, retrieval test day. This suggests that disrupted vHP processing during extinction learning led to weak extinction, and thus high fear. As increased vHP inhibition initially impaired fear retrieval memory, extinction may require hippocampal recall of fear memory and the violation of the expectation of shock in order for learning of this changed association to occur. This may reflect impaired signaling and lack of plasticity between the hippocampus and the PFC during extinction learning, and thus impaired inhibition of inappropriate fear during

retrieval. Overall, these results demonstrate that the ventral hippocampus is involved in both context fear retrieval and extinction learning, and that stimulating SST interneurons disrupts both these processes.

It is also possible that the increased freezing during the extinction retrieval test could reflect context-dependency learning during extinction, rather than just impaired extinction. Extinction is highly context-dependent, and testing recall of cued fear extinction in a context different than where extinction was learned leads to an increase, or renewal, of fear (Bouton et al., 2006). While we took efforts to minimize visible light cues and keep the presence of the patch cable consistent across days, the optogenetic stimulation itself might have acted as a learned context for extinction. Continuous 20 Hz (15ms) stimulation of SST interneurons over an entire context retrieval test is hardly a normal physiological state and may have contributed to a unique internal representation of context. Thus, the absence of stimulation on the retrieval test day may have constituted a context shift "back" to the originally perceived conditioning context and the renewal of fear. We cannot fully rule out this possible influence.

In the second experiment, stimulating PFC SST interneurons did not influence freezing behavior in context fear recall or extinction recall. Given our previous finding that vHP projections to IL were preferentially activated during extinction recall, and the involvement of both PL and IL in cued fear and extinction respectively, we had hypothesized a potential influence of SST stimulation. The PFC injections were aimed at IL but did extend into some of the PL, however the fiber placement was ventrally located in IL. If anything, we expected SST interneuron stimulation, and resulting local inhibition, to result in impaired extinction recall due to IL's role in extinction of cued fear. Our results would suggest that PFC SST interneurons stimulation is not sufficient to cause a change in freezing behavior during either fear or extinction recall of contextual fear.

We did see higher freezing in the same ChR2 mice in a tone fear conditioning paradigm during the initial stimulation of tone extinction and in a stimulation free extinction retrieval test, replicating previous work from Do-Monte et al and showing that our stimulation was effective. Most of the literature demonstrating PFC involvement in extinction utilizes auditory tone conditioning. Work from Roger Clem's lab has shown that optogenetic manipulation of SST interneurons in the PL does influence auditory cued fear. Optogenetic inhibition of SST interneurons in PL reduced freezing when paired with the auditory CS, and stimulation caused freezing in the absence of a CS (Cummings and Clem, 2020; Cummings et al., 2022). Given that the PL is thought to promote fear, they explain this unexpected direction of the effect by demonstrating that SST stimulation caused disinhibition of parvalbumin interneurons in the PL. Whether or not our manipulation affected PV cells, we did not see any change in freezing during context fear recall or extinction recall. We based our optogenetic stimulation on Clem's parameters, using 20Hz 15ms pulse stimulation since they demonstrated that 20Hz stimulation in 20ms and 5ms bursts both affect freezing in PL. One difference is the length of stimulation. During auditory paradigms, stimulation is paired with a 20-30 second tone, whereas we stimulated across 3-minute periods or the entire context retrieval test (6 minutes). While stimulating interneurons over minutes may cause different effects than over 20 seconds, this was effective in the vHP to impair fear memory and extinction learning. Overall, it is possible that contextual conditioning and extinction engage the IL differently from cued conditioning. As opposed to being one factor to help interpret whether or not to be afraid of a once fearful discrete cue, our results suggest that extinction of fear purely to a context is less influenced by IL activity.

However, one important discrepancy in these studies is that our stimulation of PFC interneurons was done during extinction recall, rather than during extinction learning like

in the auditory fear paradigm and in the vHP manipulation. This provides a good comparison to the c-Fos activity data in Chapter 2, which assessed activity during context recall, but leaves the impact of IL on context extinction learning an open question. The last experiment sought to test if increased inhibition of the IL would impair extinction learning in a similar manner to the vHP, but this experiment failed to have any effect on auditory fear extinction and seems not to have been effective due to low viral infection rates. Thus, it is possible that the IL may still be needed for context extinction learning, but not for extinction recall.

Overall, these experiments demonstrate a broad role for the vHP in both expressing context fear and learning to suppress fear during extinction, while the PFC does not appear to be necessary for context fear or extinction expression but is necessary for extinction of fear for discrete cues. The hippocampus is often focused on for its role in spatial memory and forming contextual representations. These results support the role of the hippocampus as generating and communicating contextual information to other fear related regions, while also showing its bidirectional influences on fear behavior. On the other hand, these results suggest that PFC activity is not necessary for context fear or extinction behavior, highlighting a difference in how this region influences fear to discrete cues versus contextual fear. These results give us a better understanding of how these regions influence fear and extinction behavior, and how they may fit into the broader fear circuit to interpret contextual information involved in fear learning, whether that context is the subject or background for the learned fear association.

Chapter 5: General Discussion

The hippocampus combines features of an environment into a conjunctive contextual representation, which is further capable of encoding episodic content such as the valence of fear and extinction memories. Though prior research has made it clear that the hippocampus maintains distinct representations for fear and extinction memories of a singular context (Lacagnina et al., 2019), how these different memory representations activate different behavioral states is not fully understood. Neurons in the ventral hippocampal (vHP) CA1 and subiculum project to other brain regions involved in fear and extinction recall, and investigation of activity in this region can elucidate the hippocampus's role in conveying contextual memory to a broader fear circuit to modulate behavior. The research described in this dissertation has shown that the vHP recruits different projection pathways during fear and extinction recall, and that inhibiting the vHP impacts both context fear recall and extinction learning. These results give further evidence that the hippocampus encodes the valence of contextual memory and can modulate recall and behavior through selective activation of projections to other regions.

In Chapter 2, we show that fear and extinction recall differentially activate vHP projection neurons. Using CTb to retrogradely label vHP neurons projecting to IL or BLA, and c-Fos as a marker of recent cellular activity, we see that after fear recall there is more c-Fos activity in vHP neurons projecting to BLA than those projecting to IL, and a shift after extinction to higher activity in neurons projecting to IL rather than BLA. It's important to note that both of these projections show activity in both recall events, but the ratio of activity between these populations changes. In the pre-exposure experiment, we show that context learning prior to fear conditioning does not result in high activity in IL projections like seen after extinction recall, however activity is relatively even amongst vHP projections to IL and BLA in this condition, suggesting some recruitment of this

pathway. These results provide evidence that the ventral hippocampus is sensitive to the valence of the associational context memory, and activity changes to potentially route information to regions involved in valence relevant recall and behavior.

To test the influence of these projections on memory and behavior directly, we tried to selectively inhibit vHP projections. Based on our results in Chapter 2, vHP projections to BLA are more active during fear recall and projections to IL are more active during extinction recall, and if these projections are indeed necessary, inhibiting these projections should impair memory during recall tests. In Chapter 3 we employed intersectional optogenetic and chemogenetic strategies to selectively inhibit vHP projections. These experiments did not show effects of these manipulations and raised more questions than they answered. Rather than showing a true negative result, these experiments may not have produced strong inhibition of these pathways due to methodological and behavioral issues. We had some concerns over whether there was strong enough inhibition, due to placement of optic fibers and sparce viral labeling of neurons. It could also be the case that our manipulations were effective, and that inhibition of vHP projections to BLA do not impair context fear retrieval and inhibition of vHP projections to IL do not impair context fear extinction retrieval. As multiple other studies have shown that the vHP projections to BLA are necessary for retrieval of contextual fear, the conclusion that in our hands this projection is not necessary is hard to believe. However, it is possible that the vHP projection to IL is not necessary for the retrieval of context extinction. The IL could be a necessary region for context-dependent control over ambiguous fear situations. Little evidence exists on how the IL impacts contextual fear extinction, and it is possible that context extinction memory can be expressed while bypassing the IL. However, these experiments inspired us to think about other ways of inhibiting the vHP and to critically analyze all aspects of our results.

In Chapter 4, we used optogenetic stimulation of SST interneurons to induce inhibition within the vHP and PFC. Inhibiting the vHP in this manner during fear retrieval impaired recall and reduced fear behavior. Inhibiting the vHP during extinction trials initially showed the same effect, but when testing extinction recall without inhibition we saw an increase in fear compared to controls, suggesting an impairment in extinction learning. A few different conclusions can be drawn from this result. Perhaps inhibiting the vHP is preventing the proper memory formation for extinction and disrupting plasticity in vHP synapses that may underlie extinction (Peters et al., 2010; Rosas-Vidal et al., 2018). This suggests that the hippocampus is involved in both fear and extinction, potentially through a recruitment of different projections to influence expression and suppression of fear. This interpretation would support the hippocampal role of maintaining distinct memories for fear and safety rather than conveying neutral contextual information. On the other hand, this result also suggests that recall of the context fear memory, along with the violation of the expectation of shock, is needed for proper extinction learning. As these experiments generally increase inhibition in the vHP rather than specific projection, it's possible that both modification of extinction and fear memories, and plasticity changes in both projections to IL and BLA, could be necessary for extinction learning.

Interestingly, stimulating SST interneurons to inhibit IL did not influence context fear or extinction, but demonstrated IL necessity during auditory fear extinction. This suggests that the IL may be more involved in mediating cued fear extinction rather than context extinction. However, if the vHP projection to the IL is activated during context extinction as demonstrated in Chapter 2, what is the influence of this activity? It's possible that contextual information is conveyed, but inhibiting the IL is not sufficient to impair extinction recall, which would be in line with our results from Chapter 3. While it has been shown that different hippocampal projections to the basal and central amygdala mediate

context fear and contextually-gated cued fear, less is known about this how contextual information is conveyed from the vHP to the IL and what influence it has on behavior. It is possible that the PFC is only necessary for extinction of cue-associated fear, and not contextual fear. The PFC may play an executive function role in mediating fear, considering contextual cues and updated memory to determine how predictive a tone cue may be at that instance. Perhaps context memory itself, being so entwined with hippocampal episodic memory in general, contains enough information about emotional valence to not require such a PFC mediator. Additionally, the PFC may still be required for learning of extinction, but not the expression of extinction memory. Further tests focused on IL inhibition during context extinction learning would better replicate the vHP results and provide more understanding of the dynamics between these two regions during context extinction.

These experiments all aimed to investigate how hippocampal representations of context memory can activate different behavioral states, but teasing apart behavior and memory is not trivial. Freezing, an ethological behavior to avoid detections by predators, is a good measure of fear, but it is often the only measure we use in the lab. You can block freezing by lesioning the hippocampus, which we believe is memory related, but you can also block freezing by manipulating hypothalamic regions that activate defensive responses. Where does memory stop, and behavior begin? For instance, it's possible that our stimulation of SST interneurons in the vHP for 6 straight minutes could be causing odd, non-physiological sensory and autonomic effects. Perhaps mice are freezing not because of recalled fear, but confusion or lack of ability to move properly. Assessing other behavioral correlates of fear and running control behavioral tests to assess locomotion could substantiate some of these results. Similarly, differences in learning vs expressing fear and extinction should be carefully considered in future studies, particularly in

understanding the PFCs contribution to extinction. Additionally, maybe PFC activity could reflect processing of the level of absolute fear experienced, rather than memory per se. There is much still to investigate about how these brain regions work in tandem to give rise to complex thought, feeling, and behavior.

Together, this work advances our understanding of how the hippocampus processes memories of opposing valence and conveys contextual fear and extinction memory to other brain regions. These experiments demonstrate that in the ventral CA1 and subiculum, where projections are located to signal to other fear and extinction related regions, hippocampal activity is sensitive to changing valence of contextual memory and can influence both fear and extinction behavior. The hippocampus has long been thought of as a structure that can organize specific experience within a contextual map and guide behavior (Tolman, 1948; Schiller et al., 2015). Contextual fear memory requires spatial memory as well as emotional and behavioral regulation, incorporating hippocampal processing across all subregions, from dorsal to ventral pole. The separate learned associations of fear and extinction within a single context are distinctly encoded (Lacagnina et al., 2019), and this work further shows that the ventral hippocampus differentially represents opposing valanced memory through activation of projections to relevant brain regions involved in specific recall and behavior, and can influence both fear and extinction behavior.

References

Amano, T., Unal, C. T., and Paré, D. (2010). Synaptic correlates of fear extinction in the amygdala. *Nat. Neurosci.* 13, 489–494. doi: 10.1038/nn.2499.

Amaral, D. G., and Witter, M. P. (1989). The three-dimensional organization of the hippocampal formation: A review of anatomical data. *Neuroscience* 31, 571–591. doi: 10.1016/0306-4522(89)90424-7.

Anacker, C., Luna, V. M., Stevens, G. S., Millette, A., Shores, R., Jimenez, J. C., et al. (2018). Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. *Nature*, 1. doi: 10.1038/s41586-018-0262-4.

Andersen, P., Bliss, T. V. P., and Skrede, K. K. (1971). Lamellar organization of hippocampal excitatory pathways. *Exp. Brain Res.* 13, 222–238. doi: 10.1007/BF00234087.

Arruda-Carvalho, M., and Clem, R. L. (2014). Pathway-selective adjustment of prefrontal-amygdala transmission during fear encoding. *J. Neurosci.* 34, 15601–15609. doi: 10.1523/JNEUROSCI.2664-14.2014.

Bach, D. R., Guitart-Masip, M., Packard, P. A., Miró, J., Falip, M., Fuentemilla, L., et al. (2014). Human hippocampus arbitrates approach-avoidance conflict. *Curr. Biol.* 24, 541–547. doi: 10.1016/j.cub.2014.01.046.

Bannerman, D. M., Deacon, R. M. J., Offen, S., Friswell, J., Grubb, M., and Rawlins, J. N. P. (2002). Double dissociation of function within the hippocampus: Spatial memory and hyponeophagia. *Behav. Neurosci.* 116, 884–901. doi: 10.1037/0735-7044.116.5.884.

Bannerman, D. M., Good, M. A., Yee, B. K., Heupel, M. J., Iversen, S. D., and Rawlins, J. N. P. (1999). Double dissociation of function within the hippocampus: A comparison of dorsal, ventral, and complete hippocampal cytotoxic lesions. *Behav. Neurosci.* 113, 1170–1188. doi: 10.1037/0735-7044.113.6.1170.

Bannerman, D. M., Grubb, M., Deacon, R. M. J., Yee, B. K., Feldon, J., and Rawlins, J. N. P. (2003). Ventral hippocampal lesions affect anxiety but not spatial learning. *Behav. Brain Res.* 139, 197–213. doi: 10.1016/S0166-4328(02)00268-1.

Bannerman, D. M., Rawlins, J. N. P., McHugh, S. B., Deacon, R. M. J., Yee, B. K., Bast, T., et al. (2004). Regional dissociations within the hippocampus - Memory and anxiety. *Neurosci. Biobehav. Rev.* 28, 273–283. doi: 10.1016/j.neubiorev.2004.03.004.

Bannerman, D. M., Sprengel, R., Sanderson, D. J., Mchugh, S. B., Rawlins, J. N. P., Monyer, H., et al. (2014). Hippocampal synaptic plasticity, spatial memory and anxiety. *Nat. Rev. Neurosci.* 15, 181–192. doi: 10.1038/nrn3677.

Bast, T., Zhang, W. N., and Feldon, J. (2001). The ventral hippocampus and fear conditioning in rats: Different anterograde amnesias of fear after tetrodotoxin inactivation and infusion of the GABAA agonist muscimol. *Exp. Brain Res.* 139, 39–52. doi: 10.1007/s002210100746.

Biane, J. S., Ladow, M. A., Stefanini, F., Boddu, S. P., Fan, A., Hassan, S., et al. (2023).

Neural dynamics underlying associative learning in the dorsal and ventral hippocampus. *Nat. Neurosci.* 26, 798–809. doi: 10.1038/s41593-023-01296-6.
Bloodgood, D. W., Sugam, J. A., Holmes, A., and Kash, T. L. (2018). Fear extinction requires infralimbic cortex projections to the basolateral amygdala. *Transl. Psychiatry* 8. doi: 10.1038/s41398-018-0106-x.
Bolles, R. C. (1970). Species-specific defense reactions and avoidance learning - An evaluative review. *Psychol. Rev.* 77, 32–48. doi: 10.1007/BF03001275.
Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. *Psychol. Bull.* 114, 80–99. Available at: https://psycnet.apa.org/doi/10.1037/0033-2909.114.1.80.
Bouton, M. E., and Bolles, R. C. (1979). Contextual control of the extinction of conditioned fear. *Learn. Motiv.* 10, 445–466. doi: 10.1016/0023-9690(79)90057-2.
Bouton, M. E., and Bolles, R. C. (1980). Conditioned fear assessed by freezing and by the suppression of three different baselines. *Anim. Learn. Behav.* 8, 429–434. doi: 10.3758/BF03199629.
Bouton, M. E., and King, D. A. (1983). Contextual control of the extinction of conditioned fear: Tests for the associative value of the context. *J. Exp. Psychol. Anim. Behav. Process.* 9, 248–265. doi: https://psycnet.apa.org/doi/10.1037/0097-7403.9.3.248.
Bouton, M. E., Westbrook, R. F., Corcoran, K. A., and Maren, S. (2006). Contextual and Temporal Modulation of Extinction: Behavioral and Biological Mechanisms. *Biol. Psychiatry* 60, 352–360. doi: 10.1016/j.biopsych.2005.12.015.
Bukalo, O., Pinard, C. R., Silverstein, S., Brehm, C., Hartley, N. D., Whittle, N., et al. (2015). Prefrontal inputs to the amygdala instruct fear extinction memory formation. *Sci. Adv.* 1, 1–9. doi: 10.1126/sciadv.1500251.
Burgos-Robles, A., Vidal-Gonzalez, I., and Quirk, G. J. (2009). Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. *J. Neurosci.* 29, 8474–8482. doi: 10.1523/JNEUROSCI.0378-09.2009.
Campbell, S., Marriott, M., Nahmias, C., and MacQueen, G. M. (2004). Lower hippocampal volume in patients suffering from depression: a meta-analysis. *Am. J. Psychiatry* 161, 598–607. doi: 10.1176/appi.ajp.161.4.598.
Cenquizca, L. A., and Swanson, L. W. (2007). Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. *Brain Res. Rev.* 56, 1–26. doi: 10.1016/j.brainresrev.2007.05.002.
Chen, B. K., Murawski, N. J., Cincotta, C., McKissick, O., Finkelstein, A., Hamidi, A. B., et al. (2019). Artificially Enhancing and Suppressing Hippocampus-Mediated Memories. *Curr. Biol.* 29, 1885-1894.e4. doi: 10.1016/j.cub.2019.04.065.
Colgin, L. L., Moser, E. I., and Moser, M. B. (2008). Understanding memory through hippocampal remapping. *Trends Neurosci.* 31, 469–477. doi: 10.1016/j.tins.2008.06.008.
Corcoran, K. a, and Maren, S. (2001). Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *J. Neurosci.* 21, 1720–1726. doi:

21/5/1720 [pii].
Crawford, M., and Masterson, F. A. (1982). Species-specific defense reactions and avoidance learning - An evaluative review. *Pavlov. J. Biol. Sci. Off. J. Pavlov.* 17, 204–214. doi: 10.1007/BF03001275.
Cummings, K. A., Bayshtok, S., Dong, T. N., Kenny, P. J., and Clem, R. L. (2022). Control of fear by discrete prefrontal GABAergic populations encoding valence-specific information. *Neuron* 110, 3036-3052.e5. doi: 10.1016/j.neuron.2022.07.004.
Cummings, K. A., and Clem, R. L. (2020). Prefrontal somatostatin interneurons encode fear memory. *Nat. Neurosci.* 23, 61–74. doi: 10.1038/s41593-019-0552-7.
Davis, M. (1992). The Role Of The Amygdala In Fear And Anxiety. *Annu. Rev. Neurosci.* 15, 353–375. doi: 10.1146/annurev.neuro.15.1.353.
Denny, C. A., Kheirbek, M. A., Alba, E. L., Tanaka, K. F., Brachman, R. A., Laughman, K. B., et al. (2014). Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. *Neuron* 83, 189–201. doi: 10.1016/j.neuron.2014.05.018.
Do-Monte, F. H., Manzano-Nieves, G., Quiñones-Laracuente, K., Ramos-Medina, L., and Quirk, G. J. (2015). Revisiting the role of infralimbic cortex in fear extinction with optogenetics. *J. Neurosci.* 35, 3607–3615. doi: 10.1523/JNEUROSCI.3137-14.2015.
Dolorfo, C. L., and Amaral, D. G. (1998). Entorhinal Cortex of the Rat: Topographic Organization of the Cells of Origin of the Perforant Path Projection to the Dentate Gyrus. *J. Comp. Neurol* 398, 25–48. doi: 10.1002/(SICI)1096-9861(19980817)398:1<25::AID-CNE3>3.0.CO;2-B.
Fanselow, M. (2010). From Contextual Fear to a Dynamic View of Memory Systems. *Trends Cogn. Sci.* 14, 1–16. doi: 10.1016/j.tics.2009.10.008.From.
Fanselow, M., and Dong, H.-W. (2010). Are the Dorsal and Ventral Hippocampus functionally distinct structures. *Neuron* 65, 1–25. doi: 10.1016/j.neuron.2009.11.031.Are.
Fanselow, M. S. (1990). Factors governing one trial contextual conditioning. *Anim. Learn. Behav.* 18, 264–270.
Fanselow, M. S. (2000). Contextual fear, gestalt memories, and the hippocampus. *Behav. Brain Res.* 110, 73–81. doi: 10.1016/S0166-4328(99)00186-2.
Fanselow, M. S., and Ledoux, J. E. (1999). Why We Think Plasticity Underlying Pavlovian Fear Conditioning Occurs in the Basolateral Amygdala. *Neuron* 23, 229–232. doi: https://doi.org/10.1016/S0896-6273(00)80775-8.
Feinstein, J. S., Adolphs, R., Damasio, A., and Tranel, D. (2011). The human amygdala and the induction and experience of fear. *Curr. Biol.* 21, 34–38. doi: 10.1016/j.cub.2010.11.042.
Frankland, P. W., Bontempi, B., Talkton, L. E., Kaczmarek, L., and Silva, A. J. (2004). The Involvement of the Anterior Cingulate Cortex in Remote Contextual Fear Memory. *Science (80-.).* 304, 881–883. doi: 10.1126/science.1094804.
Gabbott, P., Headlam, A., and Busby, S. (2002). Morphological evidence that CA1 hippocampal afferents monosynaptically innervate PV-containing neurons and

NADPH-diaphorase reactive cells in the medial prefrontal cortex (Areas 25/32) of the rat. *Brain Res.* 946, 314–322. doi: 10.1016/S0006-8993(02)02487-3.
Gage, F. H., and Thompson, R. G. (1980). Differential distribution of norepinephrine and serotonin along the dorsal-ventral axis of the hippocampal formation. *Brain Res. Bull.* 5, 771–773. doi: 10.1016/0361-9230(80)90220-8.
Gale, G. D., Anagnostaras, S. G., Godsil, B. P., Mitchell, S., Nozawa, T., Sage, J. R., et al. (2004). Role of the Basolateral Amygdala in the Storage of Fear Memories across the Adult Lifetime of Rats. 24, 3810–3815. doi: 10.1523/JNEUROSCI.4100-03.2004.
Gilbertson, M. W., Shenton, M. E., Ciszewski, A., Kasai, K., Lasko, N. B., Orr, S. P., et al. (2010). Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nat. Neurosci.*, 1242–1247. doi: 10.1038/nn958.Smaller.
Giustino, T. F., Fitzgerald, P. J., and Maren, S. (2016). Fear expression suppresses medial prefrontal cortical firing in rats. *PLoS One* 11, 1–13. doi: 10.1371/journal.pone.0165256.
Graham, J., D'Ambra, A. F., Jung, S. J., Teratani-Ota, Y., Vishwakarma, N., Venkatesh, R., et al. (2021). High-Frequency Stimulation of Ventral CA1 Neurons Reduces Amygdala Activity and Inhibits Fear. *Front. Behav. Neurosci.* 15, 1–13. doi: 10.3389/fnbeh.2021.595049.
Gray, J. A., and McNaughton, N. (2000). *The neuropsychology of anxiety*. 2nd editio. Oxford University Press.
Greenburg, M., and Ziff, E. (1984). Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogeneo. *Nature* 311, 433–38. Available at: https://www.nature.com/articles/311433a0.pdf.
Grewe, B. F., Gründemann, J., Kitch, L. J., Lecoq, J. A., Parker, J. G., Marshall, J. D., et al. (2017). Neural ensemble dynamics underlying a long-term associative memory. *Nature* 543, 670–675. doi: 10.1038/nature21682.
Gruber, A. J., Hussain, R. J., and O'Donnell, P. (2009). The nucleus accumbens: A switchboard for goal-directed behaviors. *PLoS One* 4. doi: 10.1371/journal.pone.0005062.
Gruber, A. J., and McDonald, R. J. (2012). Context, emotion, and the strategic pursuit of goals: interactions among multiple brain systems controlling motivated behavior. *Front. Behav. Neurosci.* 6, 1–26. doi: 10.3389/fnbeh.2012.00050.
Harris, E., Witter, M. P., Weinstein, G., and Stewart, M. (2001). Intrinsic Connectivity of the Rat Subiculum: I. Dendritic Morphology and Patterns of Axonal Arborization by Pyramidal Neurons. *J. Comp. Neurol.* 435, 490–505.
Henke, P. G. (1990). Hippocampal pathway to the amygdala and stress ulcer development. *Brain Res. Bull.* 25, 691–695. doi: 10.1016/0361-9230(90)90044-Z.
Herman, J. P., Dolgas, C. M., and Carlson, S. L. (1998). Ventral subiculum regulates hypothalamo-pituitary-adrenocortical and behavioural responses to cognitive stressors. *Neuroscience* 86, 449–459. doi: 10.1016/S0306-4522(98)00055-4.
Herry, C., Ciocchi, S., Senn, V., Demmou, L., Müller, C., and Lüthi, A. (2008). Switching on and off fear by distinct neuronal circuits. *Nature* 454, 600–606. doi:

10.1038/nature07166.
Herry, C., Trifilieff, P., Micheau, J., Lüthi, A., and Mons, N. (2006). Extinction of auditory fear conditioning requires MAPK/ERK activation in the basolateral amygdala. *Eur. J. Neurosci.* 24, 261–269. doi: 10.1111/j.1460-9568.2006.04893.x.
Hock, B. J., and Bunsey, M. D. (1998). Differential effects of dorsal and ventral hippocampal lesions. *J. Neurosci.* 18, 7027–7032. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9712671.
Hoover, W. B., and Vertes, R. P. (2007). Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Struct. Funct.* 212, 149–179. doi: 10.1007/s00429-007-0150-4.
Huckleberry, K. A., Shue, F., Copeland, T., Chitwood, R. A., Yin, W., and Drew, M. R. (2018). Dorsal and ventral hippocampal adult-born neurons contribute to context fear memory. *Neuropsychopharmacology*, 1. doi: 10.1038/s41386-018-0109-6.
Irle, E., Ruhleder, M., Lange, C., Seidler-Brandler, U., Salzer, S., Dechent, P., et al. (2010). Reduced amygdalar and hippocampal size in adults with generalized social phobia. *J. Psychiatry Neurosci.* 35, 126–131. doi: 10.1503/jpn.090041.
Jimenez, J. C., Su, K., Goldberg, A. R., Luna, V. M., Biane, J. S., Ordek, G., et al. (2018). Anxiety Cells in a Hippocampal-Hypothalamic Circuit. *Neuron* 97, 670-683.e6. doi: 10.1016/j.neuron.2018.01.016.
Jin, J., and Maren, S. (2015). Fear renewal preferentially activates ventral hippocampal neurons projecting to both amygdala and prefrontal cortex in rats. *Sci. Rep.* 5, 24–26. doi: 10.1038/srep08388.
Josselyn, S. A. (2010). Continuing the search for the engram: Examining the mechanism of fear memories. *J. Psychiatry Neurosci.* 35, 221–228. doi: 10.1503/jpn.100015.
Josselyn, S. A., Köhler, S., and Frankland, P. W. (2015). Finding the engram. *Nat. Rev. Neurosci.* 16, 521–534. doi: 10.1038/nrn4000.
Jung, M., Wiener, S., and McNaughton, B. (1994). Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. *J. Neurosci.* 14, 7347–7356. doi: 10.1523/JNEUROSCI.14-12-07347.1994.
Kesner, R. P., and Rolls, E. T. (2015). A computational theory of hippocampal function, and tests of the theory : New developments. *Neurosci. Biobehav. Rev.* 48, 92–147. doi: 10.1016/j.neubiorev.2014.11.009.
Kheirbek, M. A., Drew, L. J., Burghardt, N. S., Costantini, D. O., Tannenholz, L., Ahmari, S. E., et al. (2014). Differential control of learning and anxiety along the dorso- ventral axis of the dentate gy. *Neuron* 77, 955–968. doi: 10.1016/j.neuron.2012.12.038.Differential.
Kim, J. J., and Fanselow, M. S. (1992). Modalilty-specific retrograde amnesia of fear. *Science (80-.).* 256, 675–677.
Kishi, T., Tsumori, T., Yokota, S., and Yasui, Y. (2006). Topographical projection from the hippocampal formation to the amygdala: A combined anterograde and retrograde tracing study in the rat. *J. Comp. Neurol.* 496, 349–368. doi: 10.1002/cne.20919.
Kjelstrup, K. G., Tuvnes, F. A., Steffenach, H.-A., Murison, R., Moser, E. I., and Moser, M.-B. (2002). Reduced fear expression after lesions of the ventral hippocampus.

Proc. Natl. Acad. Sci. 99, 10825–10830. doi: 10.1073/pnas.152112399.
Köhler, C. (1985). Intrinsic projections of the retrohippocampal region in the rat brain. 1. The subicular complex. *J. Comp. Neurol.* 236, 504–522.
Lacagnina, A. F., Brockway, E. T., Crovetti, C. R., Shue, F., McCarty, M. J., Sattler, K. P., et al. (2019). Distinct hippocampal engrams control extinction and relapse of fear memory. *Nat. Neurosci.* doi: 10.1038/s41593-019-0361-z.
Lashley, K. S. (1950). In Search of the Engram. *Soc. Exp. Biol. Physiol. Mech. Anim. Behav. (Society's Symp. IV.) Acad. Press*, 454–482.
Lattal, K. M., Radulovic, J., and Lukowiak, K. (2006). Extinction: Does It or Doesn't It? The Requirement of Altered Gene Activity and New Protein Synthesis. *Biol. Psychiatry* 60, 344–351. doi: 10.1016/j.biopsych.2006.05.038.
Laurent, V., and Westbrook, R. F. (2009). Inactivation of the infralimbic but not the prelimbic cortex impairs consolidation and retrieval of fear extinction. *Learn. Mem.* 16, 520–529. doi: 10.1101/lm.1474609.
Ledoux, J. E. (2000). Emotion Circuits in the Brain. *Annu. Rev. Neurosci.* 23, 155–184.
LeDoux, J. E. (2014). Coming to terms with fear. *Proc. Natl. Acad. Sci. U. S. A.* 111, 2871–2878. doi: 10.1073/pnas.1400335111.
LeGates, T. A., Kvarta, M. D., Tooley, J. R., Francis, T. C., Lobo, M. K., Creed, M. C., et al. (2019). Reward behavior is regulated by the strength of hippocampus-nucleus accumbens excitatory synapses. *Nature* 564, 258–262. doi: 10.1038/s41586-018-0740-8.Reward.
Leutgeb, J. K., Leutgeb, S., Moser, M. B., and Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science (80-.).* 315, 961–966. doi: 10.1126/science.1135801.
Likhtik, E., Popa, D., Apergis-Schoute, J., Fidacaro, G. A., and Paré, D. (2008). Amygdala intercalated neurons are required for expression of fear extinction. *Nature* 454, 642–645. doi: 10.1038/nature07167.
Likhtik, E., Stujenske, J. M., Topiwala, M. A., Harris, A. Z., and Gordon, J. A. (2014). Prefrontal entrainment of amygdala activity signals safety in learned fear and innate anxiety. *Nat. Neurosci.* 17, 106–113. doi: 10.1038/nn.3582.Prefrontal.
Liu, X., and Carter, A. G. (2018). Ventral hippocampal inputs preferentially drive corticocortical neurons in the infralimbic prefrontal cortex. *J. Neurosci.* 38, 7351–7363. doi: 10.1523/JNEUROSCI.0378-18.2018.
Liu, X., Ramirez, S., Pang, P. T., Puryear, C. B., Govindarajan, A., Deisseroth, K., et al. (2012a). Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature* 484, 381–385. doi: 10.1038/nature11028.
Liu, X., Ramirez, S., Pang, P. T., Puryear, C. B., Govindarajan, A., Deisseroth, K., et al. (2012b). Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature* 484, 381–385. doi: 10.1038/nature11028.
Marek, R., Jin, J., Goode, T. D., Giustino, T. F., Wang, Q., Acca, G. M., et al. (2018). Hippocampus-driven feed-forward inhibition of the prefrontal cortex mediates relapse of extinguished fear. *Nat. Neurosci.* 21, 384–392. doi: 10.1038/s41593-018-0073-9.

Maren, S., Aharonov, G., and Fanselow, M. S. (1996a). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: Absence of a temporal gradient. *Behav. Neurosci.* 110, 718–726. doi: 10.1037/0735-7044.110.4.718.

Maren, S., Aharonov, G., and Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behav. Brain Res.* 88, 261–274. doi: 10.1016/S0166-4328(97)00088-0.

Maren, S., Aharonov, G., Stote, D. L., and Fanselow, M. S. (1996b). N-methyl-D-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behav. Neurosci.* 110, 1365–1374. doi: 10.1037/0735-7044.110.6.1365.

Maren, S., and Hobin, J. A. (2007). Hippocampal regulation of context-dependent neuronal activity in the lateral amygdala. *Learn. Mem.* 14, 318–324. doi: 10.1101/lm.477007.

Maren, S., and Holt, W. G. (2004). Hippocampus and Pavlovian Fear Conditioning in Rats: Muscimol Infusions into the Ventral, but Not Dorsal, Hippocampus Impair the Acquisition of Conditional Freezing to an Auditory Conditional Stimulus. *Behav. Neurosci.* 118, 97–110. doi: 10.1037/0735-7044.118.1.97.

Maren, S., Phan, K. L., and Liberzon, I. (2013). The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat Rev Neurosci.* 14, 417–428. doi: 10.1038/nrn3492.The.

Maren, S., and Quirk, G. J. (2004). Neuronal signalling of fear memory. *Nat. Rev. Neurosci.* 5, 844–852. doi: 10.1038/nrn1535.

McDonald, R. J., Balog, R. J., Lee, J. Q., Stuart, E. E., Carrels, B. B., and Hong, N. S. (2018). Rats with ventral hippocampal damage are impaired at various forms of learning including conditioned inhibition, spatial navigation, and discriminative fear conditioning to similar contexts. *Behav. Brain Res.* 351, 138–151. doi: 10.1016/j.bbr.2018.06.003.

McDonald, R. J., Jones, J., Richards, B., and Hong, N. S. (2006). A double dissociation of dorsal and ventral hippocampal function on a learning and memory task mediated by the dorso-lateral striatum. *Eur. J. Neurosci.* 24, 1789–1801. doi: 10.1111/j.1460-9568.2006.05064.x.

McDonald, R. J., King, A. L., and Hong, N. S. (2001). Context-specific interference on reversal learning of a stimulus-response habit. *Behav. Brain Res.* 121, 149–165. doi: 10.1016/S0166-4328(01)00160-7.

McHugh, S. B., Deacon, R. M. J., Rawlins, J. N. P., and Bannerman, D. M. (2004). Amygdala and Ventral Hippocampus Contribute Differentially to Mechanisms of Fear and Anxiety. *Behav. Neurosci.* 118, 63–78. doi: 10.1037/0735-7044.118.1.63.

Milad, M. R., and Quirk, G. J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature* 420, 70–74. doi: 10.1038/nature01138.

Miller, D. B., Rassaby, M. M., Collins, K. A., and Milad, M. R. (2022). Behavioral and Neural Mechanisms of Latent Inhibition. *Learn. Mem.* 29, 38–47. doi: 10.1101/lm.053439.121.

Miller, R. R., Kasprow, W. J., and Schachtman, T. R. (1986). Retrieval Variability : Sources and Consequences. *Am. J. Psychol.* 99, 145–218.
Moita, M. A. P. (2004). Putting Fear in Its Place: Remapping of Hippocampal Place Cells during Fear Conditioning. *J. Neurosci.* 24, 7015–7023. doi: 10.1523/JNEUROSCI.5492-03.2004.
Morgan, J. I., and Curran, T. (1989). Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. *Trends Neurosci.* 12, 459–462. doi: 10.1016/0166-2236(89)90096-9.
Morgan, M. A., and LeDoux, J. E. (1995). Differential Contribution of Dorsal and Ventral Medial Prefrontal Cortex to the Acquisition and Extinction of Conditioned Fear in Rats. *Behav. Neurosci.* 109, 681–688. doi: 10.1037/0735-7044.109.4.681.
Morgan, M. A., Romanski, L. M., and LeDoux, J. E. (1993). Extinction of emotional learning: Contribution of medial prefrontal cortex. *Neurosci. Lett.* 163, 109–113. doi: 10.1016/0304-3940(93)90241-C.
Morris, R. G. M., Garrud, P., Rawlins, J. N. P., and O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature* 297, 681–683. doi: 10.1038/297681a0.
Moser, E. I., Moser, M. B., and Andersen, P. (1993). Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. *J. Neurosci.* 13, 3916–3925. doi: 10.1016/S0378-8733(97)00003-8.
Moser, M.-B., and Moser, E. I. (1998). Distributed Encoding and Retrieval of Spatial Memory in the Hippocampus. *J. Neurosci.* 18, 7535–7542.
Moser, M. B., Moser, E. I., Forrest, E., Andersen, P., and Morris, R. G. (1995). Spatial learning with a minislab in the dorsal hippocampus. *Proc. Natl. Acad. Sci.* 92, 9697–9701. doi: 10.1073/pnas.92.21.9697.
O'Keefe, J., and Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res.* 34, 171–175. doi: 10.1016/0006-8993(71)90358-1.
O'Keefe, J., and Nadel, L. (1978). *The Hippocampus as a Cognitive Map*. Oxford University Press doi: 10.1097/00005053-198003000-00018.
O'Reilly, R. C., and Rudy, J. W. (2001). Conjunctive representations in learning and memory: Principles of cortical and hippocampal function. *Psychol. Rev.* 108, 311–345. doi: https://doi.org/10.1037/0033-295X.108.2.311.
Orsini, C. A., Kim, J. H., Knapska, E., and Maren, S. (2011). Hippocampal and Prefrontal Projections to the Basal Amygdala Mediate Contextual Regulation of Fear after Extinction. *J. Neurosci.* 31, 17269–17277. doi: 10.1523/JNEUROSCI.4095-11.2011.
Pape, H. C., and Pare, D. (2010). Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiol. Rev.* 90, 419–463. doi: 10.1152/physrev.00037.2009.
Park, S., Kramer, E. E., Mercaldo, V., Rashid, A. J., Insel, N., Frankland, P. W., et al. (2016). Neuronal Allocation to a Hippocampal Engram. *Neuropsychopharmacology*

41, 2987–2993. doi: 10.1038/npp.2016.73.
Peters, J., Dieppa-Perea, L. M., Melendaz, L. M., and Quirk, G. J. (2010). Induction of Fear Extinction with Hippocampal-Infralimbic BDNF. *Science (80-.).* 328, 1288–1290. doi: 10.1126/science.1186909.
Petrovich, G. D., Canteras, N. S., and Swanson, L. W. (2001). Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. *Brain Res. Rev.* 38, 247–289. doi: 10.1016/S0165-0173(01)00080-7.
Pothuizen, H. H. J., Zhang, W. N., Jongen-Rêlo, A. L., Feldon, J., and Yee, B. K. (2004). Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: A within-subject, within-task comparison of reference and working spatial memory. *Eur. J. Neurosci.* 19, 705–712. doi: 10.1111/j.0953-816X.2004.03170.x.
Quirk, G. J., Repa, J. C., and LeDoux, J. E. (1995). Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: Parallel recordings in the freely behaving rat. *Neuron* 15, 1029–1039. doi: 10.1016/0896-6273(95)90092-6.
Quirk, G. J., Russo, G. K., Barron, J. L., and Lebron, K. (2000). The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *J. Neurosci.* 20, 6225–6231. doi: 20/16/6225 [pii].
Ramirez, S., Liu, X., Lin, P. A., Suh, J., Pignatelli, M., Redondo, R. L., et al. (2013). Creating a false memory in the hippocampus. *Science (80-.).* 341, 387–391. doi: 10.1126/science.1239073.
Rescorla, R. A. (1968). Probability of Shock in the Presence and Absence of CS in Fear Conditioning. *J. Comp. Physiol. Psychol.* 66, 1–5. doi: 10.1037/h0025984.
Rescorla, R. A. (1987). Pavlovian Conditioning. *Annu. Rev. Neurosci.* 43, 151–160.
Richmond, M. A., Pouzet, B., Veenman, L., Feldon, J., Yee, B. K., Rawlins, J. N. P., et al. (1999). Dissociating context and space within the hippocampus: Effects of complete, dorsal, and ventral excitotoxic hippocampal lesions on conditioned freezing and spatial learning. *Behav. Neurosci.* 113, 1189–1203. doi: 10.1037/0735-7044.113.6.1189.
Risold, P. Y., and Swanson, L. W. (1996). Structural evidence for functional domains in the rat hippocampus. *Science (80-.).* 272, 1484–1486. doi: 10.1126/science.272.5267.1484.
Rolls, E. T. (1996). A theory of hippocampal function in memory. *Hippocampus* 6, 601–620. doi: 10.1002/(SICI)1098-1063(1996)6:6<601::AID-HIPO5>3.0.CO;2-J.
Rolls, E. T. (2018). The storage and recall of memories in the hippocampo-cortical system. *Cell Tissue Res.* 373, 577–604. doi: 10.1007/s00441-017-2744-3.
Rosas-Vidal, L. E., Do-Monte, F. H., Sotres-Bayon, F., and Quirk, G. J. (2014). Hippocampal-prefrontal BDNF and memory for fear extinction. *Neuropsychopharmacology* 39, 2161–2169. doi: 10.1038/npp.2014.64.
Rosas-Vidal, L. E., Lozada-Miranda, V., Cantres-Rosario, Y., Vega-Medina, A., Melendez, L., and Quirk, G. J. (2018). Alteration of BDNF in the medial prefrontal cortex and the ventral hippocampus impairs extinction of avoidance. *Neuropsychopharmacology* 43, 2636–2644. doi: 10.1038/s41386-018-0176-8.

Rudy, J. W., Barrientos, R. M., and O'Reilly, R. C. (2002). Hippocampal formation supports conditioning to memory of a context. *Behav. Neurosci.* 116, 530–538. doi: 10.1037/0735-7044.116.4.530.

Schiller, D., Eichenbaum, H., Buffalo, E. A., Davachi, L., Foster, D. J., Leutgeb, S., et al. (2015). Memory and Space: Towards an Understanding of the Cognitive Map. *J. Neurosci.* 35, 13904–13911. doi: 10.1523/JNEUROSCI.2618-15.2015.

Schumacher, A., Villaruel, F. R., Ussling, A., Riaz, S., Lee, A. C. H., and Ito, R. (2018). Ventral Hippocampal CA1 and CA3 Differentially Mediate Learned Approach-Avoidance Conflict Processing. *Curr. Biol.* 28, 1318-1324.e4. doi: 10.1016/j.cub.2018.03.012.

Scoville, W. B., and Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *J. Neurol. Neurosurg. Psychiatry*, 11–21.

Sharpe, M. J., and Killcross, S. (2015). The prelimbic cortex uses higher-order cues to modulate both the acquisition and expression of conditioned fear. *Front. Syst. Neurosci.* 8, 1–10. doi: 10.3389/fnsys.2014.00235.

Shurtleff, D., and Ayres, J. J. B. (1981). One-trial backward excitatory fear conditioning in rats: Acquisition, retention, extinction, and spontaneous recovery. *Anim. Learn. Behav.* 9, 65–74. doi: 10.3758/BF03212027.

Sierra-Mercado, D., Padilla-Coreano, N., and Quirk, G. J. (2011). Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology* 36, 529–538. doi: 10.1038/npp.2010.184.

Simon, P., Dupuis, R., and Costentin, J. (1993). Thigmotaxis as an index of anxiety in mice . Influence of dopaminergic transmissions. *Behav. Brain Res.* 61, 59–64.

Soler-Cedeño, O., Torres-Rodríguez, O., Bernard, F., Maldonado, L., Hernández, A., and Porter, J. T. (2019). Plasticity of NMDA receptors at ventral hippocampal synapses in the infralimbic cortex regulates cued fear. *eNeuro* 6, 1–12. doi: 10.1523/ENEURO.0354-18.2019.

Sotres-Bayon, F., Bush, D. E. A., and LeDoux, J. E. (2007). Acquisition of fear extinction requires activation of NR2B-containing NMDA receptors in the lateral amygdala. *Neuropsychopharmacology* 32, 1929–1940. doi: 10.1038/sj.npp.1301316.

Sotres-Bayon, F., Sierra-Mercado, D., Pardilla-Delgado, E., and Quirk, G. J. (2012). Gating of fear in prelimbic cortex by hippocampal and amygdala inputs. *Neuron* 76, 804–812. doi: http://dx.doi.org/10.1016/j.neuron.2012.09.028.

Sparta, D. R., Stamatakis, A. M., Phillips, J. L., Hovelsø, N., Van Zessen, R., and Stuber, G. D. (2012). Construction of implantable optical fibers for long-term optogenetic manipulation of neural circuits. *Nat. Protoc.* 7, 12–23. doi: 10.1038/nprot.2011.413.

Stern, C. A. J., Gazarini, L., Vanvossen, A. C., Hames, M. S., and Bertoglio, L. J. (2014). Activity in prelimbic cortex subserves fear memory reconsolidation over time. *Learn. Mem.* 21, 14–20. doi: 10.1101/lm.032631.113.

Swanson, C. J., and Kalivas, P. W. (2000). Regulation of locomotor activity by metabotropic glutamate receptors in the nucleus accumbens and ventral tegmental area. *J Pharmacol Exp Ther* 292, 406–14.

Swanson, L. W., and Cowan, W. M. (1977). An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. *J. Comp. Neurol.* 172, 49–84.
Swanson, L. W., Wyss, J. M., and Cowan, W. M. (1978). An autorediographic study of the organization of intrahippocampal association pathways in the rat. *J. Comp. Neurol.* 181, 681–715.
Tamamaki, N., Abe, K., and Nojyo, Y. (1987). Columnar organization in the subiculum formed by axon branches originating from single CA1 pyramidal neurons in the rat hippocampus. *Brain Res.* 412, 156–160. doi: 10.1016/0006-8993(87)91452-1.
Taube, J. S. (2007). The Head Direction Signal: Origins and Sensory-Motor Integration. *Annu. Rev. Neurosci.* 30, 181–207. doi: 10.1146/annurev.neuro.29.051605.112854.
Tolman, E. C. (1948). Cognitive maps in rats and men. *Psychol. Rev.* 55, 189–208. doi: 10.1037/h0061626.
Trent, N. L., and Menard, J. L. (2010). The ventral hippocampus and the lateral septum work in tandem to regulate rats' open-arm exploration in the elevated plus-maze. *Physiol. Behav.* 101, 141–152. doi: 10.1016/j.physbeh.2010.04.035.
Troy Harker, K., and Whishaw, I. Q. (2004). A reaffirmation of the retrosplenial contribution to rodent navigation: Reviewing the influences of lesion, strain, and task. *Neurosci. Biobehav. Rev.* 28, 485–496. doi: 10.1016/j.neubiorev.2004.06.005.
van Groen, T., van Haren, F. J., Witter, M. P., and Groenwegen, H. J. (1986). The organization of the reciprocal connections between the subiculum and the entorhinal cortex in the cat: 1. A neuroanatomical tracing study. *J. Comp. Neurol.* 250, 485–497.
Van Groen, T., and Wyss, J. M. (1990). Extrinsic projections from area CA1 of the rat hippocampus: Olfactory, cortical, subcortical, and bilateral hippocampal formation projections. *J. Comp. Neurol.* 302, 515–528. doi: 10.1002/cne.903020308.
Vann, S. D., Brown, M. W., Erichsen, J. T., and Aggleton, J. P. (2000). Fos imaging reveals differential patterns of hippocampal and parahippocampal subfield activation in rats in response to different spatial memory tests. *J. Neurosci.* 20, 2711–2718. doi: 10.1371/journal.pone.0017840.
Vasquez, J. H., Leong, K. C., Gagliardi, C. M., Harland, B., Apicella, A. J., and Muzzio, I. A. (2019). Pathway specific activation of ventral hippocampal cells projecting to the prelimbic cortex diminishes fear renewal. *Neurobiol. Learn. Mem.* 161, 63–71. doi: 10.1016/j.nlm.2019.03.003.
Verney, C., Baulac, M., Berger, B., Alvarez, C., Vigny, A., and Helle, K. B. (1985). Morphological evidence for a dopaminergic terminal field in the hippocampal formation of young and adult rat. *Neuroscience* 14, 1039–1052. doi: 10.1016/0306-4522(85)90275-1.
Vervliet, B., Craske, M. G., and Hermans, D. (2013). Fear Extinction and Relapse: State of the Art. *Annu. Rev. Clin. Psychol.* 9, 215–248. doi: 10.1146/annurev-clinpsy-050212-185542.
Vytal, K., and Hamann, S. (2010). Neuroimaging support for discrete neural correlates of basic emotions: A voxel-based meta-analysis. *J. Cogn. Neurosci.* 22, 2864–2885.

doi: 10.1162/jocn.2009.21366.
Wang, D., Noda, Y., Tsunekawa, H., Zhou, Y., Miyazaki, M., Senzaki, K., et al. (2007). Behavioural and neurochemical features of olfactory bulbectomized rats resembling depression with comorbid anxiety. *Behav. Brain Res.* 178, 262–273. doi: 10.1016/j.bbr.2007.01.003.
Wang, M. E., Wann, E. G., Yuan, R. K., Ramos Alvarez, M. M., Stead, S. M., and Muzzio, I. A. (2012). Long-Term Stabilization of Place Cell Remapping Produced by a Fearful Experience. *J. Neurosci.* 32, 15802–15814. doi: 10.1523/JNEUROSCI.0480-12.2012.
Wang, M. E., Yuan, R. K., Keinath, A. T., Ramos Alvarez, M. M., and Muzzio, I. A. (2015). Extinction of Learned Fear Induces Hippocampal Place Cell Remapping. *J. Neurosci.* 35, 9122–9136. doi: 10.1523/JNEUROSCI.4477-14.2015.
Wassum, K. M., Ostlund, S. B., Maidment, N. T., and Balleine, B. W. (2009). Distinct opioid circuits determine the palatability and the desirability of rewarding events. *Proc. Natl. Acad. Sci.* 106, 12512–12517. doi: 10.1073/pnas.0905874106.
Wiltgen, B. J. (2006). Context Fear Learning in the Absence of the Hippocampus. *J. Neurosci.* 26, 5484–5491. doi: 10.1523/JNEUROSCI.2685-05.2006.
Witter, M. P. (1986). A survey of the anatomy of the hippocampal formation, with emphasis on the septotemporal organization of its intrinsic and extrinsic connections. *Adv. Exp. Med. Biol.* 203, 67–82.
Witter, M. P., and Amaral, D. G. (1991). Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. *J. Comp. Neurol.* 307, 437–459. doi: 10.1002/cne.903070308.
Witter, M. P., Doan, T. P., Jacobsen, B., Nilssen, E. S., and Ohara, S. (2017). Architecture of the entorhinal cortex a review of entorhinal anatomy in rodents with some comparative notes. *Front. Syst. Neurosci.* 11, 1–12. doi: 10.3389/fnsys.2017.00046.
Xu, C., Krabbe, S., Grundemann, J., Botta, P., Fadok, J. P., Osakada, F., et al. (2016). Distinct Hippocampal Pathways Mediate Dissociable Roles of Context in Memory Retrieval. *Cell* 167, 961-972.e16. doi: 10.1016/j.cell.2016.09.051.
Zelikowsky, M., Hersman, S., Chawla, M. K., Barnes, C. A., and Fanselow, M. S. (2014). Neuronal Ensembles in Amygdala, Hippocampus, and Prefrontal Cortex Track Differential Components of Contextual Fear. *J. Neurosci.* 34, 8462–8466. doi: 10.1523/JNEUROSCI.3624-13.2014.