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Know Safety, No Fear

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Abstract

Every day we are bombarded by stimuli that must be assessed for their potential for harm or benefit. Once a stimulus is learned to predict harm, it can elicit fear responses. Such learning can last a lifetime but is not always beneficial for an organism. For an organism to thrive in its environment, it must know when to engage in defensive, avoidance behaviors and when to engage in non-defensive, approach behaviors. Fear should be suppressed in situations that are not dangerous: when a novel, innocuous stimulus resembles a feared stimulus, when a feared stimulus no longer predicts harm, or when there is an option to avoid harm. A cardinal feature of anxiety disorders is the inability to suppress fear adaptively. In PTSD, for instance, learned fear is expressed inappropriately in safe situations and is resistant to extinction. In this review, we discuss mechanisms of suppressing fear responses during stimulus discrimination, fear extinction, and active avoidance, focusing on the well-studied tripartite circuit consisting of the amygdala, medial prefrontal cortex and hippocampus.

1. Introduction

When confronted with danger, organisms must mobilize the appropriate defensive response. The transfer of previously learned defensive responses to new but similar stimuli generates faster responding. Generalization of defensive behaviors can be evolutionarily advantageous, because an organism will likely survive a false alarm but not a miss. While generalization is clearly an adaptive feature of the nervous system, withholding a defensive response when a new stimulus is safe is also important. Learning to discriminate among stimuli allows an organism to adapt and update its expectations regarding potentially threatening situations. A delicate balance between generalization and discrimination is adaptive in a continuously

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changing world. Furthermore, threat expectations and the associated behavioral responses must be sensitive to changes in the predictive status of cues. Cues that once predicted harm may no longer do so; if so, learned fear or avoidance responses should extinguish so the organism can engage in other adaptive behaviors, such as foraging or mating.

Responses to danger can include reflexes, such as freezing or fleeing an imminent threat, and active responses, such as learned avoidance responses. Active avoidance behaviors are defined as those that prevent the threat from occurring, such as lever pressing to prevent footshock. These behaviors can be difficult to modulate or extinguish once established. If an organism has removed itself from a dangerous cue or context, it may have no opportunity to observe that the cue is no longer dangerous; the organism thus misses the opportunity to acquire a new “safe” association. Furthermore, successfully avoiding a threat may itself elicit a sense of safety, thereby strengthening the avoidance behavior. Avoidance behaviors that persist long after a threat has ceased to exist are maladaptive because they unnecessarily reduce an animal’s opportunities to pursue rewards, such as social interaction, food, and mating.

Despite their immense theoretical and translational relevance, the mechanisms for distinguishing safety from fear are still unclear and their neural circuits continue to be mapped. The well-studied forebrain circuit comprising the amygdala, medial prefrontal cortex, and hippocampus has been the focus of much of the research on regulating fear responses. Here, we outline our current understanding about how this circuit mediates several forms of fear suppression, namely fear generalization versus discrimination, fear extinction, and active avoidance.

Amygdalo-cortical-hippocampal circuit

Cortical and thalamic afferents sending sensory information about a conditional stimulus (CS) and unconditional stimulus (US) converge onto principal neurons in the lateral amygdala (LA) (reviewed in ^{1,2}). The spatiotemporal association of the CS and US triggers Hebbian learning. Information is then routed from the LA to the basal amygdala (BA), which in turn projects to the central amygdala (CeA) (reviewed in ²), either directly or indirectly via a group of GABAergic intercalated cells (ITC) ³. The BA also sends and receives projections from multiple brain areas involved in valence encoding, and is often described as an integration hub within the amygdala (reviewed in ⁴). In addition to the BA, the LA also sends projections directly to the CeA to mediate conditioned fear responses ⁵. The CeA is subdivided into lateral and medial zones based on connectivity, neurochemistry, and functionality (reviewed in ²). The lateral division of the CeA (CeL) gates output from the medial division of the CeA (CeM) ⁶, which projects to downstream hypothalamic and brainstem targets that orchestrate defensive responses ⁷. Control over these defensive fear responses by the medial prefrontal cortex (mPFC) is directly mediated by bi-directional connectivity with the LA, BA and CeA ⁸. Using retrograde tracing, it was determined that there is extensive connectivity from the prelimbic region of the prefrontal cortex (PL) to the infralimbic region of the prefrontal cortex (IL), with only sparse connectivity from the IL to the PL ⁹. When the PL projections to the IL were optogenetically activated during fear extinction, faster extinction acquisition and better extinction recall was observed ⁹.

Bidirectional communication between amygdala and hippocampus has been shown to be important for establishing contextual representations (reviewed in ¹⁰ and discussed further below). While much of the research on fear expression and suppression has been seated in the dorsal hippocampus (dHIPP) (reviewed in ¹¹), the amygdala and PFC do not receive direct dHIPP projections. Instead, the amygdala receives direct projections from ventral hippocampus (vHIPP) ¹², and the PFC receives direct projections from vHIPP and intermediate hippocampus ^{13,14}.

2. Learning safety through discrimination

A. Behavioral processes

The study of stimulus discrimination and generalization is highly pertinent in the context of danger. Once a stimulus (e.g. a cue or context) has been conditioned, generalization describes the transfer of conditioned responding to stimuli that may resemble stimuli that were present during learning. Since stimuli rarely stay the same from one encounter to the next, generalization provides the organism with a mechanism to adapt in a continuously changing environment ^{15,16}. Generalization is thought of as a fundamental property of conditioning, has been computationally modeled, and even proposed as a universal law in the field of psychological science ¹⁷.

Fear memory generalization can be evaluated by pairing one particular tone frequency stimulus with a US during training, and afterwards, testing with an array of tone stimuli with novel frequencies (Figure 1A). Strong generalization is inferred when responding to the novel stimulus equals responding to the conditioned stimulus. This type of paradigm has been used for establishing generalization gradients ¹⁸. Another method for testing generalization is to train animals to associate only one stimulus (CS+) with a US, while another stimulus (CS-) that differs perceptually from CS+, is unpaired from the US (Figure 1B). Responding to the CS+ and CS- is then typically tested the next day. Generalization is inferred when responding to the CS+ and CS- is similar, and discrimination when CS+ responding is greater than CS- responding. This paradigm is often used to study safety learning and memory.

Safety learning is usually studied with either a within-subjects design, in which each subject is exposed to both the CS+ and CS- (e.g. ^{19,20}), or using a between-subjects design in which one group learns the CS+ association (fear group) and a separate group learns the CS- association (safety group) (e.g. ^{21,22}) (Figure 1C). Both approaches teach the animal that the CS- is a safety cue and animals consequently exhibit more safety behaviors during the CS- (e.g. decreased freezing, increased grooming, etc.). However, the approaches differ in how the safety groups learn about danger. In a between-subjects design, the “safety” group associates the context with harm because unsignaled shocks are presented outside of the CS- presentation. While the “safety” group is learning to discriminate between a dangerous context and a safety cue (CS-), the “fear” group is learning that the CS+ is dangerous and the background context is safe ²³. Both the safety and fear groups are learning to discriminate context from cue by assigning danger or safety values to them. A within-subjects design, on the other hand, pits a discrete fear cue against a discrete safety cue, resulting in animals discriminating between the two cues to guide their fear and safety

behaviors. Interestingly, fear to the background context can be low in this design indicating context fear is unnecessary to establish the CS– as a safety cue^{24–26}. As the safety learning field moves forward, it will be important to keep in mind the nuanced differences between these procedures and how they may impact conclusions regarding safety cue discrimination.

Below we describe results from both fear generalization and “safety” learning paradigms. We focus on the amygdalo-cortico-hippocampal (tripartite) circuit, as it is the most studied circuit for cued and contextual fear suppression.

B. Brain substrates

Amygdala

Lateral amygdala: In one influential model, the lateral amygdala (LA) is a key locus for the establishment of fear conditioning (reviewed in¹). Information is routed from the LA through the basal amygdala (BA) and central nucleus of the amygdala (CeA) to downstream hypothalamic and brainstem nuclei to initiate defensive behaviors. There are now several studies implicating the LA in the discrimination and generalization of auditory CSs^{21,27–29}. One of the most reliable methods for increasing fear generalization is by increasing the intensity of the US. For example, Ghosh and Chattarji showed more fear generalization to a novel tone after increasing the intensity of the US, which was associated with the emergence of LA cells responsive to the novel (generalized) tone²⁷. A source of these new “generalization cells” were cells formerly responsive to only the CS+, suggesting that the formation of fear generalization tips the balance of LA cells from being selectively CS responsive towards being responsive to a wider range of stimuli. In another study, discrimination of a novel tone was associated with less overlap of activated cells in the LA as compared with generalization, indicating behavioral discrimination was associated with a new population of neurons specific to well-discriminated stimuli²⁸. When these cells were chemogenetically deleted, generalization emerged, supporting a role for separable populations of LA cells in discriminating tones that resemble, but do not match, the CS²⁸. Together, these studies support a role for the encoding of both generalization and discrimination at the level of distinct cellular populations in the LA.

Generalization and discrimination also appear to be encoded at the level of LA synapses. Ostroff et al²¹ showed that fear conditioned rats possessed larger synapses on spines that previously did not contain a spine apparatus, whereas safety conditioned rats had smaller synapses. A similar bidirectional effect of fear versus safety learning has been observed in LA field potentials. Rogan et al²⁹ compared CS evoked field potentials in fear- vs. safety-conditioned mice and found that both the slope and amplitude of the evoked field potentials were increased in fear but decreased in safety conditioned mice. Together, these data show that changes within the LA are correlated with differential expression of cued fear or safety behaviors.

Overall, distinct populations of neurons in the LA segregate generalization and discrimination. Importantly, it appears that synaptic plasticity in the LA underlies transitioning from fear discrimination to generalization.

Basal amygdala: The BA is often described as an integration hub within the amygdala (reviewed in ⁴). Within the BA, recording studies further support this region in integrating and discriminating between fear and safety cues. Using a discriminative safety conditioning procedure in which each animal learns a fear cue, a safety cue and a reward cue, Sangha and colleagues have begun mapping out how the rat BA discriminates among fear, safety and reward cues. Behaviorally, these rats typically show high freezing to the fear cue and suppressed freezing when the fear and safety cues are presented together (i.e. a compound fear+safety cue). While recording BA neurons during this safety procedure, separate subpopulations of BA neurons were parsed based on how they responded to each of these cues ¹⁹. Some BA neurons responded selectively during the compound fear+safety cue where suppressed freezing was observed, in addition to when the safety cue was presented alone. These neurons seem to represent a ‘safety-specific’ microcircuit within the BA. Other neurons showed similar discriminative responding to just the fear or reward cues. Some neurons showed changes in firing to the onset of every cue presented, which may represent BA encoding of salient events regardless of valence. Finally, and perhaps most interestingly, a separate subpopulation within the BA responded to the safety and reward cues, but not the fear cue, supporting the hypothesis that safety cues engage neuronal circuits encoding reward ³⁰. Many of the BA neurons that responded to the safety and not the fear cues, became responsive to the fear cue following fear extinction ³¹. That is, some of the neurons responding to a learned safety cue showed a similar response to an extinguished fear cue, suggesting that rats perceived the extinguished fear cue as safe.

The idea that individual neurons in both the BA and LA can encode specific valences that then mediate distinct behavioral responses (reviewed in ⁴) has been recently challenged. When Kyriazi et al ³² analyzed single unit responses in the BA and LA based on CS neural responding versus conditioned behavioral responding, they observed that distributed coding across the ensemble activity of BA and LA neurons better correlated with valence information. Taken together, these data reveal the BA as a complex integration site separating and comparing multiple aspects of the cue and conditioned behavior.

Central amygdala: Classically, the central nucleus of the amygdala (CeA) was thought of as a node for information transfer from LA and BA to downstream targets. Contemporary research supports a more sophisticated role for the CeA in orchestrating defensive responses, including the modulation of fear memory generalization ^{6,33–35}. In a pioneering series of experiments, Cioocchi et al. ⁶ demonstrated that the CeM is under tonic inhibitory control by the lateral division of the CeA (CeL) and reducing tonic activity in the CeM correlated with increased generalization, whereas increasing tonic CeL activity was associated with discrimination. GABAergic CeL “off” cells mostly expressed the delta isoform of a calcium-activated protein kinase (PKC- δ^+) ³⁶. Supporting a role of CeL PKC- δ^+ “off” cells in fear generalization, photostimulation of these cells resulted in greater responding to a CS– (generalization) ³³. In further support of a role for CeA GABAergic PKC δ^+ “off” cells in fear memory generalization, α 5-GABA receptor-mediated extra-synaptic inhibition knock-down specifically on PKC δ^+ cells in the CeA resulted in enhanced fear memory generalization ³³. These data support a modulatory role for extra-synaptic α 5-GABA receptors in PKC δ^+ cells in the CeA and fear memory generalization.

In addition to the discussed role for PKC δ^+ cells in fear memory generalization, another distinct population of cells in the CeA are selective for fear discrimination³⁴. These cells contain corticotropin releasing factor (CRF) and are selectively responsive to the CS+ at low to medium, but not high, US intensities. CeL CRF knockdown impaired discrimination and the addition of CRF in the CeA enhanced discrimination. It is evident thus far that, like in the LA, CeA contains subpopulations of cells that are differentially responsive to fear discrimination and generalization. However, unlike the LA, a circuit-level understanding of fear discrimination and generalization in the CeA is far more developed.

Concerning modulatory neurotransmission in the CeA, dopaminergic (DA) signaling has been linked with both cued fear memory generalization and discrimination³⁵. Blockade of DA D2 receptors in the CeA increased generalization, while activation of DA terminals in the CeA reversed memory generalization³⁵. In addition, inhibiting DA terminals in the CeA during cued fear memory discrimination promoted generalization. These data indicate DA signaling at D2 receptors in the CeA as critical for improving the discrimination of cued fear responses. As dopamine signaling at D2 receptors inhibits cellular activity, these data support other results using global GABA knockdown methods^{34,37–40}, showing a lack of inhibitory control promotes fear generalization.

Overall, data from the CeA indicate that intermingled, yet genetically and functionally dichotomous, cells gate memory specificity and generalization in the CeA. In particular, it appears that a distinct subpopulation of GABAergic cells in the CeA (PKC δ^+) mediates fear generalization, while another population of CRH $^+$ cells in the CeA mediate fear discrimination. Finally, dopamine signaling at D2 receptors in the CeA is required for maintaining fear memory discrimination. Because the CeA is downstream from LA, BA and cortical structures involved in fear encoding, it is uniquely positioned to trigger generalized or discriminative responses based on environmental demands.

Medial prefrontal cortex—The mPFC exerts top-down control over conditioned responses and has been implicated in both the expression and inhibition of cued fear^{41–43}, as well as stimulus discrimination and generalization^{18,28,44–47}. In the mPFC, viral-mediated knock-down of key transcription regulators underlying synaptic plasticity (cAMP response element binding protein (CREB) and histone acetyltransferase binding protein (CBP)), have been associated with fear memory generalization⁴⁴. Viral-mediated deletion of the NR1 subunit of the NMDA receptor on excitatory neurons in the mPFC has also been shown to reduce the ability to control responding to the CS–⁴⁵. Together, these studies identify the mPFC as a key locus in cued fear memory accuracy and suggest a reduction in mPFC plasticity underlies cued fear memory generalization.

In rodents, the mPFC is subdivided into infralimbic (IL) and prelimbic (PL) regions, which have been shown to exhibit dissociable roles in cued fear responding^{42,43}, with the IL implicated in fear suppression and PL implicated in fear expression⁴⁸. According to this model, a modulatory role for the IL and PL over discriminative and generalized defensive responses is likely. In support, increased cued fear memory generalization over time was associated with a reduction in the activity-regulated cytoskeletal protein Arc/arg 3.1 in the IL, while discrimination over time was associated with an increase in Arc expression in the

prelimbic cortex (PL) ¹⁸. In support of a role for the PL in cued fear discrimination, Grosso et al. showed a subset of new neurons emerged in response to the presentation of a novel tone stimulus following conditioning, indicating cell-specific discrimination in the PL ²⁸. Recently, it was shown that chronic alcohol magnifies fear memory generalization and overgeneralization was associated with reduced expression of Arc in the IL ⁴⁶. Chemogenetic stimulation of the IL completely reversed the effects of alcohol on fear memory generalization, implicating activity in the IL with discrimination (fear suppression) ⁴⁶.

Dissociable roles of the PL and IL have also been observed during discrimination among fear, safety and reward cues ⁴⁷. Pharmacological inactivation of IL impaired the expression of learned safety (increased cue-induced freezing), whereas inactivation of PL did not. Instead, PL inactivation impaired discriminatory reward seeking; that is, rats did not show increased reward seeking during the learned reward cue compared to the non-reward cues. These results point to both the IL and PL in modulating cue discrimination. This idea is consistent with a recent study by Corches et al ⁴⁹ in which mice learned a specific context was associated with a CS+, CS-, or neither a cue nor footshock. Generalized fear was greater early in training compared to late in training. During generalized fear, PL neurons were activated during conditioned and generalized stimuli, whereas IL neurons were activated during learned inhibition of generalized fear ⁴⁹. Overall, an accumulation of evidence points to modulatory, and potentially dissociable roles, for the PL and IL in fear generalization and discrimination.

Hippocampus—The hippocampus generates a representation of the context in which trauma occurred, and this representation is used to activate a fear response when the same or similar context is later encountered. This process is known as contextual fear conditioning and depends on the hippocampus' ability to rapidly generate multimodal, conjunctive representations that link together multiple features of a place or context ^{50,51}. Perturbations to both dorsal and ventral hippocampus impair acquisition and expression of such context-evoked fear ^{52–54} (reviewed in ¹¹). Because excessive generalization of learned contextual fear is believed to contribute to anxiety and stress-related disorders ^{55–59}, there has been great interest in understanding the hippocampal mechanisms regulating the generalization and specificity of contextual fear memories.

The ability to distinguish among similar but distinct contexts is believed to depend on hippocampal pattern separation. Originally defined as a computational process, pattern separation refers to the ability of the dentate gyrus (DG) to orthogonalize inputs to the hippocampus—that is, to map overlapping neural inputs onto more distinct neural ensembles ^{60,61}. The ability of DG to pattern separate is believed to arise because DG has more neurons and a lower firing rate than its input structure, the entorhinal cortex. These features enhance the ability of DG to assign unique ensemble codes to different stimulus representations. Consistent with a pattern separation function, DG activity seems to be more sensitive to subtle changes in the environment as compared to other hippocampal subregions. For instance, DG place cells more readily remap in response to subtle contextual changes than place cells in CA3 ⁶². Furthermore, when rodents are exposed to several different

environments, DG granule cells typically fire in only a single environment⁶³, whereas other places cells in CA3 and CA1 fire in multiple environments.

There is strong evidence that DG pattern separation constrains generalization of contextual fear and other hippocampus-dependent memories. Seminal work by Kesner and colleagues demonstrated that lesions to DG impair rodents' ability to distinguish among similar contexts, spatial locations, or sequences^{64,65}. For instance, rats with DG lesions were impaired in their ability to distinguish two nearby locations on a Barnes maze but were unimpaired in the ability to distinguish locations that were far apart from each other⁶⁶. Perturbations of DG also impair specificity of contextual fear memory. McHugh et al.⁶⁷ showed that knocking out NMDA receptors specifically in DG impaired the ability of mice to learn a contextual fear discrimination in which the mice received alternating exposures to similar shock-paired and safe contexts. The DG KO mice froze excessively in the safe context, consistent with overgeneralization of contextual fear.

The hippocampal DG is one of a small number of brain regions that retains the ability to produce neurons throughout life in mammals, including humans^{68–70}. The process of adult neurogenesis may be important for contextualizing fear and preventing excessive generalization of learned fear. Experimental ablation of adult hippocampal neurogenesis impairs context fear conditioning in mice^{54,71,72}, as well as context fear discrimination in mice^{73,74}. Moreover, artificially boosting neurogenesis by preventing apoptosis of newborn neurons improves context fear discrimination⁷⁵. The circuit mechanisms for these effects are still unclear, but there is some evidence that neurogenesis promotes sparse coding of contexts within DG^{74,76}, thereby supporting the pattern separation function of DG (reviewed in⁷⁷).

From a clinical perspective, it is important to know whether impaired DG function necessarily leads to fear generalization. If memory specificity is impaired because of DG dysfunction, are the effects specific to fear memories, or might safety memories and other non-aversive memories also be generalized? As noted above, Kesner and colleagues have shown that DG lesions impair spatial and contextual discrimination learning in both aversive and reward-motivated tasks, suggesting that the behavioral deficits are not specific to fear. In a study that used computational modeling and behavioral testing to test theories about the contribution of DG to memory acquisition and retrieval, Bernier et al.⁷⁸ showed that the effects of DG silencing on context fear memory depend on the prior history of the animal. When DG was silenced in mice re-exposed to a shock-paired context, there was no effect on fear memory expression, demonstrating that DG is not required for context fear recall. However, if mice were exposed to similar safe and shock-paired contexts, then silencing DG during re-exposure to the shocked context reduced freezing. Computational simulations suggested that the impaired freezing under the latter condition occurred because of an impaired ability of mice to distinguish between the shock-paired context and safe contexts. As a result, mice incorrectly retrieved the safe context memory during re-exposure to the shocked context. These findings suggest that impairments to DG do not inexorably lead to excessive fear; the consequences of DG dysfunction will instead depend on the history of the animal and, in particular, on the composition of stored memories.

Dorsal (dHIPP) and ventral (vHIPP) hippocampus are functionally differentiated, with dorsal typically associated with cognitive functions such as spatial navigation and ventral hippocampus associated with regulation of emotional states such as fear and anxiety^{79,80}. For this reason, it seems likely that dorsal and ventral hippocampus make distinct contributions to contextual fear, but there are scant data explicitly addressing this issue. The hippocampus-amygdala and hippocampus-PFC projections that are required for hippocampal regulation of fear expression arise from the ventral hippocampus^{81,82}. Nevertheless, perturbations of either dorsal or ventral hippocampus are sufficient to impair contextual fear, suggesting that contextual fear memory is, in some sense, distributed across the entire dorsal-ventral axis of the hippocampus^{52,53}. Based on evidence that dorsal hippocampus generates more precise spatial representations than ventral hippocampus^{83,84}, one can speculate that ventral hippocampus contextualizes emotional memories in a more general, “vicinity-based” way, whereas dorsal hippocampus associates emotions with a specific, well-defined place. To our knowledge, this idea has not been tested. A second unknown is how contextual information encoded in dorsal hippocampus reaches the amygdala projections arising from the ventral hippocampus. Dorsal-to-ventral communication could be mediated through longitudinal projections within hippocampus or through extra-hippocampal regions such as entorhinal cortex⁸⁵.

C. Summary

Fidelity of fear memory expression is controlled by a distributed brain network that includes the amygdala, mPFC, hippocampus and other regions not discussed here. The mechanisms of fear regulation within the amygdala, mPFC and hippocampus appear to be quite different. Within the amygdala, expression, suppression and generalization of fear are mediated by molecularly and/or morphologically distinct subpopulations of cells. At the level of the CeA, discrimination and generalization of cued fear is gated by distinct subpopulations of neurons. In the BA, there are also distinct subpopulations of neurons responding to specific learned cues representing fear and safety. At the level of the LA, neurons specific to generalization seem to emerge upon presentation of a stimulus that triggers behavioral generalization, indicating that generalization is a form of learning-induced plasticity in the LA. Through reciprocal projections between these amygdalar subnuclei and the mPFC, the IL and PL may be modulating the direction of generalized versus discriminative fear. By contrast, in the hippocampus, fear expression, generalization and discrimination appear to all arise from the same general-purpose pattern separation and pattern completion mechanisms. Although hippocampal memory generalization can be actively regulated⁸⁶, generalization of hippocampal fear memory is often seen as representing a failure of pattern separation rather than the engagement of active generalization mechanisms. However, recent discoveries about hippocampal mechanisms of memory linking⁸⁷ call for a reevaluation of this view. This work suggests that, like amygdala, hippocampus contains unique mechanisms that adaptively modulate fear discrimination and generalization.

Thus far we have discussed the neural mechanisms of fear generalization and discrimination, using fear cues that always predict shock, and safety cues that always represent the absence of shock. We now turn our attention to how fear is suppressed during cues that once signaled danger but no longer predict an aversive outcome.

3. Learning safety through extinction

A. Behavioral processes

Extinction was first described by Pavlov⁸⁸, using an “alimentary” conditioning paradigm, in which conditioned stimuli were paired with the presentation of food or acid, both of which elicited salivation as the unconditioned response (UR). Pavlov showed that the conditioned response (CR) declined in strength when the CS was repeatedly presented without the US. The term “extinction” has been used to describe both the procedure (presentations of the CS without the US) and the behavioral phenomenon (loss of the CR). Exposure therapy, a common behavioral therapy for anxiety and stress-related disorders, uses extinction to reduce maladaptive learned fear^{55,57}. Deficits in fear extinction have been hypothesized to contribute to disorders such as PTSD^{20,89}.

One of Pavlov’s most influential conclusions was that extinction reflects neither a wholesale loss of the CS-US association nor fatigue of the sensory or motor systems. This conclusion was based on evidence that the CR could return after extinction without additional training, which was termed spontaneous recovery⁸⁸. This indicates that extinction does not abolish the associative link between the CS and US. Pavlov also observed that the CR could be evoked after extinction by presentation of the US alone prior to re-presenting the CS. This phenomenon, later termed reinstatement⁸⁹, meant that extinction was not caused by simple fatigue of the sensory or motor systems. Bouton and colleagues⁹⁰ revealed a third type of CR recovery after extinction, when they demonstrated that extinction exhibits context specificity, meaning that the effects of an extinction treatment are strongest in the place in which the extinction treatment took place. When a subject is moved to a new context after extinction, the CR recovered, a phenomenon called “renewal.” All three recovery phenomena are observed in fear conditioning and have important implications with respect to our understanding of fear regulation. First, fear recovery demonstrates that extinction does not completely abolish the original fear memory. Instead, extinction involves new inhibitory learning that presumably recruits neural mechanisms beyond those involved in fear acquisition. Second, fear recovery presents a conundrum in the clinical domain to the extent that it curtails the effectiveness of extinction-based treatments. In the following sections, we will discuss extinguishing contextual versus cue-based fears.

To extinguish cued fear, the CS is simply presented repeatedly without the US, which can happen successfully regardless of whether the animal is in the original fear context or a new neutral context. To extinguish contextual fear, the animal is re-exposed to the fear-associated context and the US is not presented. The neural circuitry and molecular mechanisms involved in fear extinction have already been reviewed in depth in several recent reviews (e.g. ^{10,81,91–93}). Here we will provide a brief summary, highlighting findings within the amygdalo-cortico-hippocampal circuit.

B. Brain substrates

Amygdala—Acquisition of fear extinction requires NR2B-containing NMDA receptors in the lateral amygdala⁹⁴. However, extinction can also induce depotentiation at LA synapses that were potentiated after fear conditioning⁹⁵, indicating decreased activity within the LA

may be associated with extinction. If this simple scenario were true, it would follow that decreased activity within the BA would also be associated with extinction, but it is not that simple. While collecting single unit recordings from the mouse BA during cued fear conditioning and extinction, Herry and colleagues⁴¹ identified two classes of neurons that correlated with high and low fear states: ‘fear’ neurons and ‘extinction’ neurons. ‘fear’ neurons were characterized by showing an excitatory response to the fear cue during fear conditioning and at the beginning of extinction training, when fear behavior was high. Extinction training reduced the excitatory response in these ‘fear’ neurons, leading to a CS-evoked inhibition instead. ‘Extinction’ neurons were found to emerge as extinction training progressed as fear behavior progressively declined. These neurons did not show any cue-elicited activity during fear conditioning or at the beginning of extinction when fear behavior was high, but when fear was reduced by extinction training, these neurons began to show increased responding to the cue. These results strongly suggest that neurons in the BA are contributing to the acquisition of extinction learning to suppress conditioned fear.

These BA ‘extinction’ neurons may be providing increased input to a cluster of GABAergic cells that lie between the LA/BA and CeA, the intercalated cells (ITC). These cells are required for the expression of fear extinction⁹⁶ and provide increased inhibition onto CeA fear output neurons after extinction⁹⁷. Consistent with this, fear extinction has also been associated with increased expression in the CeA of the GAD65 isoform of the GABA-synthesizing enzyme glutamic acid decarboxylase⁹⁸. The collective literature suggests that fear extinction can impact signaling at multiple levels within the amygdala, ultimately resulting in decreased output by the medial division of the CeA.

Medial prefrontal cortex—Several studies have investigated the role of IL during fear extinction, particularly cued fear extinction. Recordings and inactivations isolated to the IL region have indicated that IL activity is needed for both consolidation and recall of cued fear extinction. Early reports showed IL neurons increasing in activity during extinction recall one day after extinction acquisition training, but not during the acquisition phase itself⁹⁹. However, when the IL region was electrically stimulated with the cue during extinction acquisition, better extinction acquisition and recall one day later was seen, indicating IL activity during acquisition can influence the strength of the extinction memory^{99,100}. Conversely, when the IL was pharmacologically inactivated during extinction acquisition, the extinction acquisition curve was negatively affected and recall the next day was impaired⁴⁸. Interestingly, when IL inactivation was limited to the consolidation phase during relearning of extinction, poor memory for extinction was observed during recall¹⁰¹. Together these data provide strong evidence that IL is needed for consolidating extinction. IL inactivation limited to extinction recall has also been shown to impair expression of learned extinction, as well as expression of learned safety^{47,101} (but see¹⁰²), implicating IL’s necessity in recalling extinction.

In more recent studies, specific inputs and outputs of the IL have been selectively investigated during fear extinction. During cued fear conditioning, theta coupling was found to be high between the LA and the IL early in extinction acquisition, when fear behavior was also high, but reduced across extinction acquisition and partially rebounded during extinction recall¹⁰³. It is possible that these changes in theta coupling may functionally

strengthen the connections between LA and IL neurons to gate cued fear. Increased activity was also observed in BA neurons targeting IL, specifically after extinction, and optogenetically silencing this projection during fear extinction led to weaker extinction¹⁰⁴. When the IL input to the BLA (LA+BA combined) was optogenetically increased or decreased during extinction acquisition, Bukalo et al¹⁰⁵ observed no differences in the extinction acquisition curve but did see bidirectional effects on later extinction recall: increased IL input resulted in better extinction recall and, conversely, decreased IL input resulted in less extinction recall. When the authors manipulated IL's input to the BLA during recall, they saw no effect on extinction recall, indicating IL->BLA input is driving consolidation of fear extinction memory and not the expression or recall of learned extinction. Complementary to these findings, rats that discriminated between a CS+ and CS- showed increased theta synchronization between the BLA and mPFC during the CS-, with BLA activity being entrained to the theta input from the mPFC during the CS-¹⁰⁶. These data seem to suggest that bidirectional IL-BLA signaling is more crucial during acquisition of fear suppression, and other projections from the IL, such as those to the CeA or ITC, may be mediating the recall of learned fear suppression.

Just dorsal to the IL, lies the PL which appears to play a role in fear expression. When the PL was inactivated during extinction training, fear expression was reduced⁴⁸. However, the next day under drug-free conditions, these animals still expressed memory for the extinction training. Similar results were seen when the PL was inactivated during the relearning of extinction: reduced fear expression during PL inactivation but normal expression of extinction the next day when tested drug-free¹⁰¹. These results would suggest that PL activity is not necessary to learn extinction, which would be in line with earlier results showing no changes in PL activity during extinction training⁹⁹. Furthermore, PL recordings made during a discriminative fear conditioning task found that PL neurons were activated during both conditioned and generalized stimuli⁴⁹, suggesting that PL activity may be driving behavior towards generalization, which would impede fear suppression. Fittingly, when recordings were made in both the PL and IL within the same animal, the relative balance of PL and IL activity correlated with expressed fear behavior: when PL activity was higher than IL activity, higher levels of fear were seen¹⁰⁷.

Hippocampus—The hippocampus plays at least two seemingly distinct roles in fear extinction. One role of the hippocampus is to mediate the influence of contextual cues in guiding when and where to suppress learned fear. After extinction of auditory fear conditioning, the context is a powerful cue that determines whether the auditory cue will predict shock or not¹⁰⁸. If recall occurs in the same context as extinction, reduced fear is usually observed (i.e. good extinction recall), but if recall occurs in the original fear conditioning context or a novel context, high fear is typically observed (i.e. renewal). Marek et al¹⁰⁹ were able to block this context-mediated fear relapse by inhibiting the input from the ventral hippocampus (vHIPP) to IL. They showed that the vHIPP sends glutamatergic projections onto IL interneurons which then provide feedforward inhibition to IL pyramidal neurons, presumably those projecting to the amygdala. This would indicate that increased vHIPP->IL signaling would increase fear expression. Indeed, the authors showed they could block extinction recall by driving this input. These findings are consistent with earlier

findings that showed pharmacologically inactivating the vHIPP during extinction training reduced fear expression during training, with no evidence of extinction memory the next day⁴⁸.

The second major role of hippocampus in fear extinction concerns extinction of contextual fear. When rodents are presented with pairings between a tone CS and footshock, they typically acquire fear of both the tone and the physical context in which the pairings took place¹¹⁰. Such contextual fear conditioning (CFC) requires plasticity in the hippocampus, which is necessary for generating a conjunctive representation of the context^{111,112} that binds together multiple features from different sensory modalities⁵⁰. Like tone fear extinction, context fear extinction requires integrity of the mPFC¹⁰¹. However, there is also evidence that contextual fear extinction recruits hippocampal mechanisms that are not required for tone fear extinction.

The standard view of CFC and other forms of classical conditioning is that conditioning establishes an association between the CS and US, such that subsequent exposures to the CS cause reactivation of the US representation. In CFC, hippocampus is hypothesized to generate a contextual representation, which, after context-shock pairings, acquires the ability to evoke fear through hippocampal projections to amygdala⁵¹. Implicit in this view is the idea that conditioning and extinction do not alter the hippocampal context representation—they merely modify the ability of the context representation to activate fear. This model is supported by behavioral experiments demonstrating that context memory formation and context-shock learning are separable processes¹¹³, that rodents readily fear condition to remembered context representations¹¹⁴, and that fear conditioning can be induced by pairing artificial reactivation of a hippocampal context representation with shock¹¹⁵.

Recent work suggests, however, that the hippocampus is not merely a passive spectator during fear conditioning and extinction, and, indeed, plasticity of hippocampal context representations may contribute to both CFC acquisition and extinction. Electrophysiological recordings in hippocampal CA1 show that fear conditioning and extinction alter place representations. Moita et al¹¹⁶ recorded from CA1 place cells as rats explored 2 arenas before and after fear conditioning to one of the arenas. Place cell maps in the control (non-conditioned) arena remained stable across recording sessions, but in the conditioned arena, place cells remapped, meaning that place fields appeared, disappeared, or changed location. Wang et al.¹¹⁷ confirmed this finding and demonstrated that new place maps induced by fear conditioning remained stable for at least several days. Another study by the Muzzio group¹¹⁸ demonstrated that fear extinction also induced place field remapping, suggesting that place cell ensembles code emotional valence in addition to the spatial properties of an environment. Because ensemble patterns of place cell activity are believed to contribute to hippocampal context representations, the remapping caused by fear conditioning and extinction suggests shifts in context valence alter hippocampal context representations.

Do these shifts in hippocampal context representations play a causal role in fear attenuation during extinction? Optogenetic silencing of DG impairs acquisition of fear extinction but not expression of either fear or extinction memory, indicating that the hippocampus is necessary for establishing an extinction memory⁷⁸. Tronson et al¹¹⁹ showed that context fear

acquisition and extinction, respectively, evoke c-Fos and pErk expression in separate populations of CA1 neurons, suggesting they recruit distinct hippocampal ensembles. Lacagnina et al.¹²⁰ directly examined hippocampal coding of extinction using activity dependent neural tagging. The Arc-CreERT2 system was used to tag DG neurons activated during either fear acquisition or fear extinction. Immediate-early gene expression indicated that extinction-tagged neurons were most highly reactivated 5 days after extinction when fear was low, whereas acquisition-tagged neurons were reactivated 28 days after extinction, when fear had spontaneously recovered. Furthermore, optogenetic silencing of extinction-tagged cells increased fear expression after extinction, whereas silencing of acquisition-tagged neurons prevented spontaneous recovery. These findings suggest that extinction of contextual fear is mediated by the creation of a new hippocampal representation of the context as “safe.”

However, another study suggests that reactivation of hippocampal fear neurons also plays a role in extinction. Khalaf et al.¹²¹ found that extinction of a remote fear memory (i.e., one acquired about 1 month before extinction) reactivated the same neurons that are reactivated during fear recall. In this study, reactivation of fear recall neurons was found to predict extinction success. Furthermore, ablation of recall-induced neurons impaired extinction, whereas pharmacogenetic activation of these neurons enhanced extinction.

Why did the Lacagnina et al.¹²⁰ and Khalaf et al.¹²¹ studies reach different conclusions about the role of hippocampal context fear representations in extinction? One critical difference between the two studies is that Khalaf compared neurons active during fear extinction to those active during fear recall, whereas Lacagnina compared extinction to fear acquisition¹²². Because the fear recall test is also an extinction session, the neurons tagged by Khalaf might have included a mixture of fear acquisition and fear extinction neurons. A second important difference is that Khalaf examined extinction of a remote fear memory (one acquired at least 28d before extinction), whereas Lacagnina examined extinction of a recently-acquired fear memory (acquired 1d before extinction). The neural mechanisms of context fear expression and extinction change over time after acquisition^{123,124}, raising the possibility that extinction of a recent fear memory transforms hippocampal context representations, whereas remote memory extinction does not.

Although the mechanisms of contextual fear extinction are just beginning to be elucidated, research in this domain provides several pointers that should inform thinking about extinction in general. In particular, CFC extinction research suggests that extinction does not solely involve learning to inhibit the fear response—extinction may also modify CS representations. Within the hippocampus, extinction seems to suppress the neural patterns associated with fear acquisition and cause the contextual CS to evoke new neural patterns, at least for recently-acquired context fear memories. Extinction may thus reduce fear, at least in part, by suppressing the extent to which the original fear CS representation is recalled. There is some evidence that this mechanism of extinction is not unique to CFC. Song et al.¹²⁵ found that lesions to auditory cortex impaired extinction but not acquisition of fear to a tone CS, suggesting that extinction required plasticity within the cortical areas that represent the auditory CS. Similarly, visual cortical lesions impair extinction to a visual fear CSs in some cases^{126,127}. Olfactory fear conditioning potentiates the synaptic output of olfactory

sensory neurons¹²⁸, raising the as-yet-unanswered question of whether extinction reverses this potentiation. These findings demonstrate that fear conditioning and fear extinction are not mediated exclusively by increases and decreases in strength of the association between CS and US representations; conditioning and extinction also modify the CS representations themselves.

C. Summary

Research on the neural mechanisms of extinction largely supports Pavlov's theory that extinction is a form of inhibitory learning rather than unlearning of the original conditioned response. Extinction training recruits plasticity in IL, a region whose activity suppresses fear but plays no obvious role in acquisition of the original fear. Moreover, both amygdala and hippocampus contain discrete neuronal populations corresponding to distinct fear and extinction memories. Whether these fear inhibitory mechanisms control fear extinction in all situations is unclear. For instance, many of the aforementioned studies of extinction mechanisms used one or a small number of extinction sessions. There is some evidence that more extensive extinction training involves depotentiation, which yields a more permanent suppression of learned fear¹²⁹. Similarly, behavioral and pharmacological interventions such as retrieval-extinction¹³⁰, immediate extinction^{131,132}, pharmacological blockade of reconsolidation^{133,134}, and epigenetic manipulations¹³⁵ may more effectively suppress fear than traditional extinction training. However, many questions remain about the generality, effectiveness, and mechanisms of these procedures (for review see¹³⁶). In summary, an extensive literature demonstrates the existence of an amygdalo-cortical-hippocampal network that suppresses fear after extinction training. It is doubtful though that these mechanisms explain all extinction phenomena. In particular, additional research is needed to determine whether some training procedures can produce a more permanent loss of fear by undoing the original fear learning.

4. Learning safety through active avoidance

A. Behavioral processes

Preventing harmful encounters requires adaptive behavioral responses. Once an organism learns that a specific environmental cue or context will lead to a dangerous outcome, the organism may choose safety if the option is available. There are two ways in which an organism can move to safety: by fleeing an immediate danger (a reactive escape response), or by preventing the occurrence of the threat through a proactive avoidance response. Active avoidance contrasts with passive avoidance, in which organisms avoid a threat by withholding (rather than performing) a response. Decades of research have examined active avoidance with various tasks, including wheel running avoidance^{137,138}, lever press avoidance^{139–141}, and shuttle avoidance^{142–145}.

The most commonly used avoidance task is shuttle avoidance. During signaled shuttle avoidance, rats learn to terminate a CS and prevent the occurrence of a US (footshock) by moving or "shuttling" between two compartments^{142–144}. Initially, rats learn that the CS predicts the occurrence of the shock US, and increased freezing to the CS is observed. Next, rats learn to escape the shock by running to the adjacent compartment. Finally, rats learn that

moving to the opposite chamber early during the CS period prevents the occurrence of shock while also terminating the CS. Thus, animals successfully learn to actively avoid the US by moving or shuttling prior its occurrence. This active avoidance response results in a reduction in freezing as the rats are trained in shuttle avoidance. This phenomenon has historically been explained by a two-factor theory of avoidance, in which fear is first acquired via Pavlovian conditioning early during shuttle training, and then avoidance is subsequently acquired via instrumental conditioning at the end of shuttle training^{146,147}. A well-known issue in the field is how the animal interprets the CS after learning to perform the shuttle response. When avoidance is successful, the CS no longer predicts shock. Why, then, do animals continue to perform the avoidance response? Are they still fearful of the CS? Some theories have suggested that the termination of the CS signifies safety^{148–151}; however, it is unclear whether CS termination represents safety per se, since the animals must shuttle into a compartment in which it was previously shocked. Recent reviews on avoidance discussing these concerns suggest that understanding the neural circuits governing the separate learning stages of avoidance may resolve these issues^{148,150}.

A recent modification of the auditory fear conditioning paradigm allows direct comparison between passive (Pavlovian) and active (operant) threat responses. In this task, rats can avoid a tone-signaled footshock by stepping onto a nearby platform¹⁵². This task provides a unique opportunity to compare the neural circuits of active avoidance and auditory fear conditioning because the same cues and context are employed, with the addition of a safety zone for the rat to avoid danger. In contrast to the shuttle avoidance task, however, the platform avoidance task incorporates a small conflict between seeking food reward and avoiding danger. Throughout the platform avoidance task, rats press a bar for sucrose pellets on a VI-30 schedule, and when the tone is presented, rats must choose to forgo food-seeking to avoid the imminent shock (stepping on the platform in the opposite corner) or continue food seeking and experience the footshock. This aspect of the task makes platform-mediated avoidance is a promising preclinical model because patients excessively avoid at the cost of goal-directed behaviors (reviewed in this issue¹⁵³).

Other paradigms have also pitted reward seeking against threat avoidance with greater magnitudes of behavioral conflict. Such tasks are ideal for studying behaviors in natural settings. For example, an animal may be foraging for food outside of its nest when it suddenly detects a predator nearby. In this scenario, the animal must decide to continue foraging for food because it is hungry, or actively avoid the predator to prevent death. Kim and colleagues have developed a dynamic laboratory paradigm in which rats retrieve sucrose pellets in the presence of a robot that mimics the movements of a predator¹⁵⁴. This paradigm allows the researcher to examine interactions between appetitive and aversive behaviors, such as foraging for food in an open arena where the animal encounters a “predator”, causing the animal to flee to a safe nesting area. What are the neural circuits governing active avoidance and how might they differ among these tasks? In the following sections, we will discuss the neural substrates that guide avoidance behaviors under various conditions.

B. Brain substrates

Amygdala—Like fear conditioning, avoidance conditioning also requires activity in the amygdala. Activity in the basolateral amygdala (BLA: BA+LA combined) has been correlated with lever-press avoidance¹⁵⁵ and is necessary for acquiring shuttle^{154,156–158} and wheel-running avoidance¹⁵⁹. Following acquisition of platform-mediated avoidance, pharmacological inactivations of the BLA have blocked avoidance expression¹⁵². Interestingly, cFos and lesion studies support the need for prefrontal suppression of the central amygdala in allowing the shuttle response to occur^{154,160}. Taken together, these studies demonstrate that the amygdala is a crucial hub needed to learn and express active avoidance.

Recent studies have begun examining the role of the amygdala under conflict, such as when an individual must choose to avoid danger or continue foraging for food. Such studies are useful for understanding the function of BLA under realistic conditions. Using the aforementioned “robogator” task, developed by Kim and colleagues¹⁵⁴, permanent lesions or transient pharmacological inactivations in rats led to increased foraging behavior and reduced defensive behavior in response to the robotic predator. Single unit recordings under the same conditions revealed synchronous activity between LA and PL cell pairs that correlated with either approach of the robotic predator during foraging (PL to LA activity) or with escape from the robotic predator after foraging (LA to PL activity)¹⁶¹. This suggests that a PL to LA pathway drives approach behavior, whereas a separate LA to PL pathway drives fleeing to safety.

In another study assessing prefrontal-amygdala interactions under conflict, neural activity of BLA neurons projecting to PL was recorded during conflicting presentations of learned aversive vs. rewarding cues¹⁶². Most BLA neurons projecting to PL exhibited excitatory responses during the aversive cue. During conflict, the activity of these neurons reliably predicted the rats’ behavior (reward seeking or freezing). This suggests that BLA neurons projecting to PL discriminate fear-associated cues from reward-associated cues to guide appropriate behavior. This pathway was further tested using optogenetics to show that photoexciting the BLA-PL pathway drives fear responses, whereas photo-inhibiting the same pathway suppresses fear responses. These findings agree with results from the robogator studies showing that amygdala-prefrontal projections drive fear responses (freezing or fleeing), and also suggest that these same projections may need to be inhibited to guide other behaviors such as active avoidance. Indeed, preliminary studies using optogenetics to map the circuits of platform-mediated avoidance show that photosilencing BLA-PL projections does not affect avoidance¹⁶³. Which BLA projections might drive active avoidance? Using the shuttle avoidance task, it was found that pharmacological inactivation of BLA projections to the ventral striatum (VS) blocked the shuttle avoidance response¹⁶⁴. Consistent with these findings, preliminary results using the platform-mediated avoidance task show that photosilencing BLA projections to VS impaired active avoidance¹⁶⁵. Together, these findings support that amygdala inputs to the VS are necessary for the expression of active avoidance.

Medial prefrontal cortex—Many studies have demonstrated the role of the mPFC in modulating avoidance responses. For instance, in shuttle avoidance, cFos studies have shown that IL activation is correlated with the expression of shuttle avoidance¹⁵⁷, and IL lesions impair its expression¹⁶⁰. Studies using lever press avoidance have also shown activation of both PL and IL during the expression of active avoidance¹⁵⁵. Furthermore, platform-mediated avoidance was disrupted following PL inactivation^{152,166}, but IL inactivation had no effect¹⁵². To further probe the role of PL in platform avoidance, single unit activity was recorded in PL in rats that underwent avoidance training or fear conditioning¹⁶⁶. Increased PL activity was correlated with freezing following fear conditioning^{162,167,168}, but decreased activity at tone onset was only observed in avoidance-trained rats, suggesting that PL inhibition is correlated with avoidability¹⁶⁶. Moreover, increased activity in the PL-BLA pathway has been correlated with increased platform avoidance behavior, as measured by cFos density of specific PL projections to BLA¹⁶⁹. Ongoing studies are currently addressing which targets of PL are necessary for avoidance using an optogenetic approach¹⁶⁵. The discrepancies in the role of specific mPFC subregions on avoidance responses is likely due to the differing demands and context of each task; specifically, that active avoidance is not associated with a specific location in the shuttle task, whereas the avoidance response competes with food-seeking in the platform task (see this issue¹⁷⁰).

If rats learned to associate a discrete cue with safety or were able to avoid the shock-associated cue, it is possible that increased PL-BLA activity would correlate with safety or active avoidance. This is consistent with the idea that prefrontal-amygdala pathways are active during safety^{106,171}, and that activating PL-BLA projections drive active avoidance^{165,169}. Another possibility is that PL is signaling safety through its projections to the CeA. With regard to active avoidance, studies using the shuttle avoidance task have shown that the mPFC suppresses CeA output to enable the shuttle response^{160,172} (reviewed in^{148,150}).

Based on the aforementioned studies that combined both learned aversive cues with rewarding cues, it is possible that neural activity during a learned cue may represent sensory-related information about the cue or its association with threat, safety, or reward. Indeed, single unit recording studies have revealed that BLA activity correlated with both sensory-related information (early during the cue presentation), as well as the behavioral response (later during the cue presentation), when a single stimulus represented reward or danger³². It is possible, therefore, that the later BLA response correlated with the behavioral action is likely signaling PL (and other targets of BLA) to carry out the appropriate behavioral action.

Hippocampus—Early studies have shown that permanent hippocampal lesions facilitate shuttle avoidance^{173–175} (reviewed in^{176,177}), whereas transient pharmacological inactivations of either dorsal or ventral hippocampus impair the expression of shuttle avoidance¹⁷⁸. Although there are no studies assessing the contribution of the hippocampus in the expression of platform-mediated avoidance, studies on the extinction of platform-mediated avoidance demonstrate that the ventral hippocampus sends BDNFergic input to both PL and IL to promote the extinction of the avoidance response¹⁷⁹. Similar to the role of the ventral hippocampus in the extinction of fear^{48,168}, the ventral hippocampus likely modulates the expression of platform avoidance by mediating behavioral flexibility.

C. Summary

Studies on active avoidance have shown the amygdalo-cortical circuitry guides behavior for avoiding harm as well as managing responses to cues with conflicting valence. The amygdala appears to be the central hub for learning and expressing avoidance responses, regardless of task, whereas the mPFC exerts top-down control to modulate the avoidance response. The activity and neural circuitry may differ, however, depending on the type of avoidance task that is utilized. For example, in shuttle avoidance, the avoidance response competes with freezing behavior, whereas in platform-mediated avoidance, freezing behavior does not compete with avoidance. Therefore, the expression of these different avoidance responses may require differential activity within the amygdala to guide the appropriate behavioral response (i.e. decreased CeA output to decrease freezing). Finally, the hippocampus also appears to play a modulatory role in avoidance; however, future studies are needed to fully characterize its contribution to the neural circuits of learned active avoidance.

5. Conclusions and Future Directions

This review focused on the mechanisms within the amygdalo-cortico-hippocampal circuit in learning safety through discrimination, extinction, and avoidance. Many of the studies discussed in this review were done exclusively in male subjects but, importantly, females are more likely to be diagnosed with an anxiety disorder¹⁸⁰. Even though studies including female subjects have been proportionally rare, several studies have reported clear sex differences in fear regulation. Human studies have shown females discriminate less between fear and safety signals than males^{181,182}. And in animal models, female mice have shown more generalization of fear to novel and safe contexts compared to males, and with this generalization, a concurrent increase in BA activity was observed¹⁸³. In a paradigm where the animal needed to discriminate among fear, safety and reward cues, Greiner et al¹⁸⁴ demonstrated clear behavioral differences between male and female rats. In this study, male and female rats showed equivalent freezing levels to the fear cue when it predicted shock. However, when the fear cue was presented with the safety cue as a compound cue indicating no shock, males showed significantly reduced freezing levels and females did not. That is, the females in this study showed a lack of learned freezing suppression. Although these findings are consistent with the majority of data pointing to reduced discrimination between fear and safety signals in females, a study by Foilb et al¹⁸⁵ did not find any differences between males and female rats in suppressing freezing.

Another study also showed similar discrimination levels in males and females early in training but then generalized fear responses in females to the safety cue with continued training¹⁸⁶. Male and female rats also respond differently to the controllability of a stressor. When male rats were exposed to a controllable shock stressor, they showed lower freezing levels one day later when briefly exposed to a different shock stressor, compared to rats that were exposed to an uncontrollable shock stressor; female rats previously exposed to a controllable shock stressor did not show this reduction in freezing¹⁸⁷. This fear reduction in males was linked to activity in PL neurons projecting to the dorsal raphe nucleus (DRN), which did not appear to be engaged in females¹⁸⁷. Interestingly, omission of a safety signal

facilitated extinction of avoidance in females, but not males¹⁸⁷. This suggests that during behavioral therapy treatments of anxiety disorders, female patients may not benefit from learning safety signals to reduce their symptoms. More research is needed in comparing differences between the sexes in regulating fear responses^{188,189}.

Taken together, the evidence suggests that when learning to suppress fear, reciprocal projections between the BLA and IL are involved in the acquisition and consolidation of fear suppression (Figure 2). Additionally, increased activity in PL neurons projecting to the IL enhances acquisition of fear extinction⁹. The later expression of this learned fear suppression does not require BLA-IL signaling, and may be mediated by IL's projections to the ITC and/or CeA instead. Increased signaling from vHIPP onto inhibitory interneurons within the PL has also been associated with better fear suppression¹⁶⁸. Projections that drive behavior towards fear expression and generalization may be PL's input to the CeA, vHIPP's input to the IL, and BLA's input to the CeA.

It is crucial that animal models of anxiety and other neuropsychiatric disorders are translatable to benefit human patients with such disorders. For example, a recent symposium brought together scientists from various research backgrounds with the purpose of integrating the results from animal, computational, and neuroimaging studies. The overarching goal was to understand the neural circuitry of maladaptive behaviors aiming to facilitate treatments for patients suffering from neuropsychiatric disorders¹⁹⁰. With regard to fear and safety learning, a human study, modeled after animal studies of fear and avoidance learning, showed that subjects who learned to actively avoid a cue-associated threat exhibited reduced threat responding compared to subjects who only underwent extinction training to the same cue-associated threat¹⁹¹. This study suggests that learning to actively avoid danger may be a more effective behavioral therapy for some individuals than extinction-based therapy.

Importantly, while the ability to regulate defensive responses is widely accepted as adaptive, inappropriately expressed defensive reactions to non-threatening or irrelevant signals is maladaptive and considered an etiological factor in the development of anxiety disorders, such as post-traumatic stress disorder (PTSD)^{192,193}. True progress in understanding the neurobiology of fear suppression will require multiple converging approaches to this question. Continued efforts to elucidate the behavioral and biological mechanisms of discriminating fear from safety, extinguishing fear to stimuli that no longer signal threat, and actively suppressing fear through avoidance will result in synergistic advances in how we treat maladaptive fear.

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References

1. LeDoux JE Emotion circuits in the brain. *Annu. Rev. Neurosci* 23, 155–84 (2000). [PubMed: 10845062]

2. Pape HC & Pare D Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiol. Rev* 90, 419–463 (2010). [PubMed: 20393190]
3. Duvarci S & Pare D Amygdala Microcircuits Controlling Learned Fear. *Neuron* 82, 966–980 (2014). [PubMed: 24908482]
4. Janak PH & Tye KM From circuits to behaviour in the amygdala. *Nature* 517, 284–292 (2015). [PubMed: 25592533]
5. Li H et al. Experience-dependent modification of a central amygdala fear circuit. *Nat. Neurosci* 16, 332–339 (2013). [PubMed: 23354330]
6. Cioocchi S et al. Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature* 468, 277–282 (2010). [PubMed: 21068837]
7. Wright KM & McDannald MA Ventrolateral periaqueductal gray neurons prioritize threat probability over fear output. *Elife* 8, 1–28 (2019).
8. Marek R, Strobel C, Bredy TW & Sah P The amygdala and medial prefrontal cortex: Partners in the fear circuit. *J. Physiol* 591, 2381–2391 (2013). [PubMed: 23420655]
9. Marek R, Xu L, Sullivan RKP & Sah P Excitatory connections between the prelimbic and infralimbic medial prefrontal cortex show a role for the prelimbic cortex in fear extinction. *Nat Neurosci* 21, 654–658 (2018). [PubMed: 29686260]
10. Maren S & Holmes A Stress and fear extinction. *Neuropsychopharmacology* 41, 58–79 (2016). [PubMed: 26105142]
11. Fanselow MS From contextual fear to a dynamic view of memory systems. *Trends Cogn. Sci* 14, 7–15 (2010). [PubMed: 19939724]
12. Pitkanen A Connectivity of the rat amygdaloid complex in *The Amygdala: A functional analysis* (ed. Aggleton JP) 31–116 (Oxford University Press, 2000).
13. Cenquizca LA & Swanson LW Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. *Brain Res. Rev* 56, 1–26 (2007). [PubMed: 17559940]
14. Jay TM & Witter MP Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris leucoagglutinin. *J. Comp. Neurol* 313, 574–586 (1991). [PubMed: 1783682]
15. Dudai Y *Memory from A to Z: keywords, concepts and beyond*. (Oxford University Press, 2002).
16. Richards BA & Frankland PW The Persistence and Transience of Memory. *Neuron* 94, 1071–1084 (2017). [PubMed: 28641107]
17. Shepard RN Toward a universal law of generalization for psychological science. *Science* 237, 1317–1323 (1987). [PubMed: 3629243]
18. Pollack GA et al. Cued fear memory generalization increases over time. *Learn. Mem* 25, 298–308 (2018). [PubMed: 29907637]
19. Sangha S, Chadick J & Janak P Safety encoding in the basal amygdala. *J. Neurosci* 33, 3744–51 (2013). [PubMed: 23447586]
20. Jovanovic T et al. Impaired fear inhibition is a biomarker of PTSD but not depression. *Depress. Anxiety* 27, 244–251 (2010). [PubMed: 20143428]
21. Ostroff LE, Cain CK, Bedont J, Monfils MH & Ledoux JE Fear and safety learning differentially affect synapse size and dendritic translation in the lateral amygdala. *Proc. Natl. Acad. Sci. U. S. A* 107, 9418–9423 (2010). [PubMed: 20439732]
22. Ronovsky M et al. A role for miR-132 in learned safety. *Sci. Rep* 9, 528 (2019). [PubMed: 30679653]
23. Pollak DD et al. An Animal Model of a Behavioral Intervention for Depression. *Neuron* 60, 149–161 (2008). [PubMed: 18940595]
24. Sangha S, Greba Q, Robinson PD, Ballendine SA & Howland JG Heightened fear in response to a safety cue and extinguished fear cue in a rat model of maternal immune activation. *Front. Behav. Neurosci* 8, 168 (2014). [PubMed: 24847231]
25. Ng KH, Pollock MW, Urbanczyk PJ & Sangha S Altering D1 receptor activity in the basolateral amygdala impairs fear suppression during a safety cue. *Neurobiol. Learn. Mem* 147, 26–34 (2018). [PubMed: 29175512]

26. Müller I, Brinkman AL, Sowinski EM & Sangha S Adolescent conditioning affects rate of adult fear, safety and reward learning during discriminative conditioning. *Sci. Rep* 8, 17315 (2018). [PubMed: 30470766]
27. Ghosh S & Chattarji S Neuronal encoding of the switch from specific to generalized fear. *Nat. Neurosci* 18, 112–120 (2015). [PubMed: 25436666]
28. Grosso A, Santoni G, Manassero E, Renna A & Sacchetti B A neuronal basis for fear discrimination in the lateral amygdala. *Nat. Commun* 9, 1214 (2018). [PubMed: 29572443]
29. Rogan MT, Leon KS, Perez DL & Kandel ER Distinct neural signatures for safety and danger in the amygdala and striatum of the mouse. *Neuron* 46, 309–20 (2005). [PubMed: 15848808]
30. Christianson JP et al. Inhibition of Fear by Learned Safety Signals: A Mini-Symposium Review. *J. Neurosci* 32, 14118–14124 (2012). [PubMed: 23055481]
31. Sangha S Plasticity of Fear and Safety Neurons of the Amygdala in Response to Fear Extinction. *Front. Behav. Neurosci* 9, 1–10 (2015). [PubMed: 25653603]
32. Kyriazi P, Headley DB & Pare D Multi-dimensional Coding by Basolateral Amygdala Neurons. *Neuron* 99, 1–14 (2018). [PubMed: 30001504]
33. Botta P et al. Regulating anxiety with extrasynaptic inhibition. *Nat Neurosci* 18, 1493–1500 (2015). [PubMed: 26322928]
34. Sanford CA et al. A Central Amygdala CRF Circuit Facilitates Learning about Weak Threats. *Neuron* 93, 164–178 (2017). [PubMed: 28017470]
35. De Bundel D et al. Dopamine D2 receptors gate generalization of conditioned threat responses through mTORC1 signaling in the extended amygdala. *Mol. Psychiatry* 21, 1545–1553 (2016). [PubMed: 26782052]
36. Haubensak W et al. Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature* 468, 270–276 (2010). [PubMed: 21068836]
37. Shaban H et al. Generalization of amygdala LTP and conditioned fear in the absence of presynaptic inhibition. *Nat. Neurosci* 9, 1028–1035 (2006). [PubMed: 16819521]
38. Bergado-Acosta JR et al. Critical role of the 65-kDa isoform of glutamic acid decarboxylase in consolidation and generalization of Pavlovian fear memory. *Learn. Mem* 15, 163–71 (2008). [PubMed: 18323571]
39. Lange MD et al. Glutamic Acid Decarboxylase 65: A Link Between GABAergic Synaptic Plasticity in the Lateral Amygdala and Conditioned Fear Generalization. *Neuropsychopharmacology* 39, 2211–2220 (2014). [PubMed: 24663011]
40. Sangha S et al. Deficiency of the 65 kDa isoform of glutamic acid decarboxylase impairs extinction of cued but not contextual fear memory. *J. Neurosci* 29, 15713–15720 (2009). [PubMed: 20016086]
41. Herry C et al. Switching on and off fear by distinct neuronal circuits. *Nature* 454, 600–606 (2008). [PubMed: 18615015]
42. Milad MR & Quirk GJ Fear extinction as a model for translational neuroscience: ten years of progress. *Annu. Rev. Psychol* 63, 129–151 (2012). [PubMed: 22129456]
43. Giustino TF & Maren S The Role of the Medial Prefrontal Cortex in the Conditioning and Extinction of Fear. *Front. Behav. Neurosci* 9, 1–20 (2015). [PubMed: 25653603]
44. Vieira PA et al. Prefrontal consolidation supports the attainment of fear memory accuracy. *Learn. Mem* 21, 394–405 (2014). [PubMed: 25031365]
45. Vieira PA et al. Prefrontal NMDA receptors expressed in excitatory neurons control fear discrimination and fear extinction. *Neurobiol. Learn. Mem* 119, 52–62 (2015). [PubMed: 25615540]
46. Scarlata MJ et al. Chemogenetic stimulation of the infralimbic cortex reverses alcohol-induced fear memory overgeneralization. *Sci. Rep* 9, 6730 (2019). [PubMed: 31040357]
47. Sangha S, Robinson P, Greba Q, Davies D & Howland J Alterations in reward, fear and safety cue discrimination after inactivation of the rat prelimbic and infralimbic cortices. *Neuropsychopharmacology* 39, 2405–13 (2014). [PubMed: 24727732]

48. Sierra-Mercado D, Padilla-Coreano N & Quirk GJ Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology* 36, 529–38 (2011). [PubMed: 20962768]
49. Corches A et al. Differential fear conditioning generates prefrontal neural ensembles of safety signals. *Behav. Brain Res* 360, 169–184 (2019). [PubMed: 30502356]
50. Huckleberry KA, Ferguson LB & Drew MR Behavioral mechanisms of context fear generalization in mice. *Learn. Mem* 23, 703–709 (2016). [PubMed: 27918275]
51. Rudy JW Context representations, context functions, and the parahippocampal-hippocampal system. *Learn. Mem* 16, 573–585 (2009). [PubMed: 19794181]
52. Matus-Amat P, Higgins E, Barrientos R & Rudy JW The Role of the Dorsal Hippocampus in the Acquisition and Retrieval of Context Memory Representations. *J. Neurosci* 24, 2431–2439 (2004). [PubMed: 15014118]
53. Rudy JW & Matus-Amat P The Ventral Hippocampus Supports a Memory Representation of Context and Contextual Fear Conditioning: Implications for a Unitary Function of the Hippocampus. *Behav. Neurosci* 119, 154–163 (2005). [PubMed: 15727521]
54. Huckleberry KA et al. Dorsal and ventral hippocampal adult-born neurons contribute to context fear memory. *Neuropsychopharmacology* 43, 2487–2496 (2018). [PubMed: 29941977]
55. Bouton ME, Mineka S & Barlow D A modern learning theory perspective on the etiology of panic disorder. *Psychol Rev* 108, 4–32 (2001). [PubMed: 11212632]
56. Kheirbek MA, Klemenhagen KC, Sahay A & Hen R Neurogenesis and generalization: a new approach to stratify and treat anxiety disorders. *Nat. Neurosci* 15, 1613–1620 (2012). [PubMed: 23187693]
57. Mineka S & Zinbarg R A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. *Am. Psychol* 61, 10–26 (2006). [PubMed: 16435973]
58. Sahay A, Wilson DA & Hen R Pattern separation: a common function for new neurons in hippocampus and olfactory bulb. *Neuron* 70, 582–588 (2011). [PubMed: 21609817]
59. Barlow D Anxiety and its disorders: the nature and treatment of anxiety and panic. (Guilford Press, 2002).
60. O'Reilly RC & McClelland JL Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off. *Hippocampus* 4, 661–682 (1994). [PubMed: 7704110]
61. Treves A & Rolls ET Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. *Hippocampus* 2, 189–199 (1992). [PubMed: 1308182]
62. Leutgeb JK, Leutgeb S, Moser M-B & Moser EI Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science* 315, 961–966 (2007). [PubMed: 17303747]
63. GoodSmith D et al. Spatial Representations of Granule Cells and Mossy Cells of the Dentate Gyrus. *Neuron* 93, 677–690.e5 (2017). [PubMed: 28132828]
64. Kesner RP An analysis of dentate gyrus function (an update). *Behav. Brain Res* 354, 84–91 (2018). [PubMed: 28756212]
65. Rolls ET & Kesner RP A computational theory of hippocampal function, and empirical tests of the theory. *Prog. Neurobiol* 79, 1–48 (2006). [PubMed: 16781044]
66. Gilbert PE, Kesner RP & Lee I Dissociating hippocampal subregions: double dissociation between dentate gyrus and CA1. *Hippocampus* 11, 626–636 (2001). [PubMed: 11811656]
67. McHugh TJ et al. Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science* 317, 94–99 (2007). [PubMed: 17556551]
68. Moreno-Jiménez EP et al. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nat. Med* 25, 554–560 (2019). [PubMed: 30911133]
69. Eriksson PS et al. Neurogenesis in the adult human hippocampus. *Nat. Med* 4, 1313–1317 (1998). [PubMed: 9809557]
70. Kempermann G et al. Human Adult Neurogenesis: Evidence and Remaining Questions. *Cell Stem Cell* 23, 25–30 (2018). [PubMed: 29681514]

71. Saxe MD et al. Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc. Natl. Acad. Sci* 103, 17501 LP–17506 (2006). [PubMed: 17088541]
72. Drew MR, Denny CA & Hen R Arrest of adult hippocampal neurogenesis in mice impairs single-but not multiple-trial contextual fear conditioning. *Behav. Neurosci* 124, 446–454 (2010). [PubMed: 20695644]
73. Tronel S et al. Adult-born neurons are necessary for extended contextual discrimination. *Hippocampus* 22, 292–298 (2012). [PubMed: 21049483]
74. Niibori Y et al. Suppression of adult neurogenesis impairs population coding of similar contexts in hippocampal CA3 region. *Nat. Commun* 3, 1253 (2012). [PubMed: 23212382]
75. Sahay A et al. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* 472, 466–470 (2011). [PubMed: 21460835]
76. Ikrar T et al. Adult neurogenesis modifies excitability of the dentate gyrus. *Front. Neural Circuits* 7, 204 (2013). [PubMed: 24421758]
77. Drew MR & Huckleberry KA Modulation of Aversive Memory by Adult Hippocampal Neurogenesis. *Neurotherapeutics* 14, 646–661 (2017). [PubMed: 28488160]
78. Bernier BE et al. Dentate Gyrus Contributes to Retrieval as well as Encoding: Evidence from Context Fear Conditioning, Recall, and Extinction. *J. Neurosci* 37, 6359–6371 (2017). [PubMed: 28546308]
79. Fanselow MS & Dong H-W Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* 65, 7–19 (2010). [PubMed: 20152109]
80. Moser MB & Moser EI Functional differentiation in the hippocampus. *Hippocampus* 8, 608–619 (1998). [PubMed: 9882018]
81. Marek R, Sun Y & Sah P Neural circuits for a top-down control of fear and extinction. *Psychopharmacology (Berl)*. 236, 313–320 (2018). [PubMed: 30215217]
82. Xu C et al. Distinct Hippocampal Pathways Mediate Dissociable Roles of Context in Memory Retrieval. *Cell* 167, 961–972.e16 (2016). [PubMed: 27773481]
83. Royer S, Sirota A, Patel J & Buzsaki G Distinct representations and theta dynamics in dorsal and ventral hippocampus. *J. Neurosci* 30, 1777–1787 (2010). [PubMed: 20130187]
84. Ruediger S, Spirig D, Donato F & Caroni P Goal-oriented searching mediated by ventral hippocampus early in trial-and-error learning. *Nat. Neurosci* 15, 1563–1571 (2012). [PubMed: 23001061]
85. Amaral DG & Witter MP The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* 31, 571–91 (1989). [PubMed: 2687721]
86. Xu W & Südhof TC A neural circuit for memory specificity and generalization. *Science* 339, 1290–5 (2013). [PubMed: 23493706]
87. Cai DJ et al. A shared neural ensemble links distinct contextual memories encoded close in time. *Nature* 534, 115–118 (2016). [PubMed: 27251287]
88. Pavlov I Conditioned reflexes. (Dover Publications, 1927).
89. Lissek S & van Meurs B Learning models of PTSD: Theoretical accounts and psychobiological evidence. *Int. J. Psychophysiol* 98, 594–605 (2015). [PubMed: 25462219]
90. Bouton ME Context and Behavioral Processes in Extinction. *Learn Mem* 11, 485–494 (2004). [PubMed: 15466298]
91. Lingawi NW, Laurent V, Westbrook RF & Holmes NM The role of the basolateral amygdala and infralimbic cortex in (re)learning extinction. *Psychopharmacology (Berl)*. 236, 303–312 (2018). [PubMed: 29959461]
92. Singewald N & Holmes A Rodent models of impaired fear extinction. *Psychopharmacology (Berl)*. 236, 21–32 (2019). [PubMed: 30377749]
93. Goode TD & Maren S Common neurocircuitry mediating drug and fear relapse in preclinical models. *Psychopharmacology (Berl)*. 236, 415–437 (2019). [PubMed: 30255379]
94. Sotres-Bayon F, Bush DEA & Ledoux JE Acquisition of Fear Extinction Requires Activation of NR2B-Containing NMDA Receptors in the Lateral Amygdala. *Neuropsychopharmacology* 32, 1929–1940 (2007). [PubMed: 17213844]

95. Kim J et al. Amygdala depotentiation and fear extinction. *Proc Natl Acad Sci U S A* 104, 20955–20960 (2007). [PubMed: 18165656]
96. Likhtik E, Popa D, Apergis-Schoute J, Fidacaro G.a. & Paré D Amygdala intercalated neurons are required for expression of fear extinction. *Nature* 454, 642–645 (2008). [PubMed: 18615014]
97. Amano T, Unal CT & Paré D Synaptic correlates of fear extinction in the amygdala. *Nat. Neurosci* 13, 489–494 (2010). [PubMed: 20208529]
98. Sangha S, Ilenseer J, Sosulina L, Lesting J & Pape H-C Differential regulation of glutamic acid decarboxylase gene expression after extinction of a recent memory vs. intermediate memory. *Learn. Mem* 19, (2012).
99. Milad MRR & Quirk GJJ Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature* 420, 70–74 (2002). [PubMed: 12422216]
100. Vidal-gonzalez I, Vidal-gonzalez B, Rauch SL & Quirk GJ Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learn. Mem* 13, 728–733 (2006). [PubMed: 17142302]
101. Laurent V & Westbrook RF Inactivation of the infralimbic but not the prelimbic cortex impairs consolidation and retrieval of fear extinction. *Learn. Mem* 16, 520–529 (2009). [PubMed: 19706835]
102. Do-Monte FH, Manzano-Nieves G, Quinones-Laracuenta K, Ramos-Medina L & Quirk GJ Revisiting the Role of Infralimbic Cortex in Fear Extinction with Optogenetics. *J. Neurosci* 35, 3607–3615 (2015). [PubMed: 25716859]
103. Lesting J et al. Patterns of coupled theta activity in amygdala-hippocampal-prefrontal cortical circuits during fear extinction. *PLoS One* 6, e21714 (2011). [PubMed: 21738775]
104. Senn V et al. Long-Range Connectivity Defines Behavioral Specificity of Amygdala Neurons. *Neuron* 81, 428–437 (2014). [PubMed: 24462103]
105. Bukalo O et al. Prefrontal inputs to the amygdala instruct fear extinction memory formation. *Sci. Adv* 1, e1500251 (2015). [PubMed: 26504902]
106. Likhtik E, Stujenske JM, Topiwala MA, Harris AZ & Gordon JA Prefrontal entrainment of amygdala activity signals safety in learned fear and innate anxiety. *Nat. Neurosci* 17, 106–113 (2014). [PubMed: 24241397]
107. Giustino TF, Fitzgerald PJ & Maren S Prefrontal Cortical Firing in Rats. *PLoS One* 11, e0165256 (2016). [PubMed: 27776157]
108. Bouton ME, Westbrook RF, Corcoran KA & Maren S Contextual and temporal modulation of extinction: behavioral and biological mechanisms. *Biol. Psychiatry* 60, 352–360 (2006). [PubMed: 16616731]
109. Marek R et al. Hippocampus-driven feed-forward inhibition of the prefrontal cortex mediates relapse of extinguished fear. *Nat. Neurosci* 21, 384–392 (2018). [PubMed: 29403033]
110. Kim JJ & Fanselow MS Modality-specific retrograde amnesia of fear. *Science* 256, 675–677 (1992). [PubMed: 1585183]
111. Fanselow MS Contextual fear, gestalt memories, and the hippocampus. *Behav. Brain Res* 110, 73–81 (2000). [PubMed: 10802305]
112. O'Reilly RC & Rudy JW Conjunctive representations in learning and memory: principles of cortical and hippocampal function. *Psychol. Rev* 108, 311–345 (2001). [PubMed: 11381832]
113. Fanselow MS Factors governing one-trial contextual conditioning. *Anim. Learn. Behav* 18, 264–270 (1990).
114. Rudy JW, Barrientos R & O'Reilly RC Hippocampal formation supports conditioning to memory of a context. *Behav Neurosci* 116, 530–538 (2002). [PubMed: 12148921]
115. Ramirez S et al. Creating a False Memory in the Hippocampus. *Science* (80-.). 341, 387–391 (2013).
116. Moita MAP, Rosis S, Zhou Y, LeDoux JE & Blair HT Hippocampal place cells acquire location-specific responses to the conditioned stimulus during auditory fear conditioning. *Neuron* 37, 485–497 (2003). [PubMed: 12575955]
117. Wang ME et al. Long-term stabilization of place cell remapping produced by a fearful experience. *J. Neurosci* 32, 15802–15814 (2012). [PubMed: 23136419]

118. Wang ME, Yuan RK, Keinath AT, Ramos Alvarez MM & Muzzio IA Extinction of Learned Fear Induces Hippocampal Place Cell Remapping. *J. Neurosci* 35, 9122–9136 (2015). [PubMed: 26085635]
119. Tronson NC et al. Segregated Populations of Hippocampal Principal CA1 Neurons Mediating Conditioning and Extinction of Contextual Fear. *J. Neurosci* 29, 3387–3394 (2009). [PubMed: 19295145]
120. Lacagnina AF et al. Distinct hippocampal engrams control extinction and relapse of fear memory. *Nat. Neurosci* 22, 753–761 (2019). [PubMed: 30936555]
121. Khalaf O et al. Reactivation of recall-induced neurons contributes to remote fear memory attenuation. 1242, 1239–1242 (2018).
122. Drew MR & Brockway ET Regulation of fear extinction and relapse by hippocampal engrams. *Neuropsychopharmacology* advanced online publication (2019).
123. Anagnostaras SG, Maren S & Fanselow MS Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *J. Neurosci* 19, 1106–1114 (1999). [PubMed: 9920672]
124. Corcoran KA, Leaderbrand K & Radulovic J Extinction of remotely acquired fear depends on an inhibitory NR2B/PKA pathway in the retrosplenial cortex. *J. Neurosci* 33, 19492–19498 (2013). [PubMed: 24336715]
125. Song EY, Boatman JA, Jung MW & Kim JJ Auditory Cortex is Important in the Extinction of Two Different Tone-Based Conditioned Fear Memories in Rats. *Front. Behav. Neurosci* 4, 24 (2010). [PubMed: 20631823]
126. Ledoux JE, Romanski L & Xagoraris A Indelibility of subcortical emotional memories. *J. Cogn. Neurosci* 1, 238–243 (1989). [PubMed: 23968507]
127. Falls WA & Davis M Visual cortex ablations do not prevent extinction of fear-potentiated startle using a visual conditioned stimulus. *Behav. Neural Biol* 60, 259–270 (1993). [PubMed: 8297322]
128. Kass MD, Rosenthal MC, Pottackal J & McGann JP Fear learning enhances neural responses to threat-predictive sensory stimuli. *Science* 342, 1389–1392 (2013). [PubMed: 24337299]
129. An B, Kim J, Park K, Lee S & Song S Amount of fear extinction changes its underlying mechanisms. *Elife* 6, e25224 (2017). [PubMed: 28671550]
130. Monfils M-H, Cowansage KK, Klann E & LeDoux JE Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories. *Science* (80-.). 324, 951–955 (2009).
131. Myers KM, Ressler KJ & Davis M Different mechanisms of fear extinction dependent on length of time since fear acquisition. *Learn. Mem* 13, 216–223 (2006). [PubMed: 16585797]
132. Bernier BE, Lacagnina AF & Drew MR Potent attenuation of context fear by extinction training contiguous with acquisition. *Learn. Mem* 22, 31–38 (2014). [PubMed: 25512575]
133. Kindt M, Soeter M & Vervliet B Beyond extinction: erasing human fear responses and preventing the return of fear. *Nat. Neurosci* 12, 256–258 (2009). [PubMed: 19219038]
134. Nader K, Schafe GE & LeDoux JE Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature* 406, 722–726 (2000). [PubMed: 10963596]
135. Stafford JM, Raybuck JD, Ryabinin A & Lattal KM Increasing Histone Acetylation in the Hippocampus-Infralimbic Network Enhances Fear Extinction. *Biol. Psychiatry* 72, 25–33 (2012).
136. Maren S Seeking a spotless mind: extinction, deconsolidation, and erasure of fear memory. *Neuron* 70, 830–845 (2011). [PubMed: 21658578]
137. Bolles R, Stokes L & Younger M Does CS termination reinforce avoidance behavior? *J. Comp. Physiol. Psychol* 62, 201 (1966). [PubMed: 5969598]
138. Gabriel M Effects of intersession delay and training level on avoidance extinction and intertrial behavior. *J. Comp. Physiol. Psychol* 66, 412–416 (1968). [PubMed: 5722050]
139. D'Amato MR & Schiff D Further Studies of Overlearning and Position Reversal Learning. *Psychol. Rep* 14, 380–382 (1964).
140. Berger D & RF B Rapid acquisition of discrete-trial lever-press avoidance: Effects of signal-shock interval. *J. Exp. Anal. Behav* 24, 227–239 (1975). [PubMed: 16811875]

141. Servatius R, Jiao X, Beck K, Pang K & Minor TR Rapid avoidance acquisition in Wistar-Kyoto rats. *Behav. Brain Res.* 2 192, 191–197 (2008).
142. Mowrer O A stimulus-response analysis of anxiety and its role as a reinforcing agent. *Psychol Rev* 46, 553–565 (1939).
143. Kamin L, Brimer C & Black A Conditioned suppression as a monitor of fear of the CS in the course of avoidance training. *J. Comp. Physiol. Psychol* 56, 497–501 (1963). [PubMed: 13962088]
144. Mowrer O Learning theory and behavior. (Wiley, 1960).
145. Bolles R Aversive control: A review of B.A. Campbell and R. M. Church's punishment and aversive behavior. *J. Exp. Anal. Behav* 16, 283–288 (1971).
146. Mowrer OH, Lamoreaux & R R. Fear as an intervening variable in avoidance conditioning. *J. Comp. Psychol* 39, 29–50 (1946). [PubMed: 21018303]
147. Levis D The case for a return to a two-factor theory of avoidance: The failure of non-fear interpretations. in *Contemporary learning theories: Pavlovian conditioning and the status of traditional learning theory* 227–277 (1989).
148. LeDoux JE, Moscarello J, Sears R & Campese V The birth, death and resurrection of avoidance: a reconceptualization of a troubled paradigm. *Mol. Psychiatry* 22, 24–36 (2017). [PubMed: 27752080]
149. Dinsmoor JA Stimuli inevitably generated by behavior that avoids electric shock are inherently reinforcing. *J. Exp. Anal. Behav* 75, 311–333 (2001). [PubMed: 11453621]
150. Cain CK Avoidance Problems Reconsidered. *Curr. Opin. Behav. Sci* 26, 9–17 (2019). [PubMed: 30984805]
151. Bolles R Species-specific defense reactions and avoidance learning. *Psychol Rev* 77, 32–48 (1970).
152. Bravo-Rivera C, Roman-Ortiz C, Brignoni-Perez E, Sotres-Bayon F & Quirk GJ Neural structures mediating expression and extinction of platform-mediated avoidance. *J. Neurosci* 34, 9736–9742 (2014). [PubMed: 25031411]
153. Diehl MM, Bravo-Rivera C & Quirk GJ The study of active avoidance: A platform for discussion. *Neurosci. Biobehav. Rev* 107, 229–237 (2019). [PubMed: 31509767]
154. Choi J-S & Kim JJ Amygdala regulates risk of predation in rats foraging in a dynamic fear environment. *Proc. Natl. Acad. Sci. U. S. A* 107, 21773–21777 (2010). [PubMed: 21115817]
155. Jiao X, Beck KD, Myers CE, Servatius RJ & Pang KCH Altered activity of the medial prefrontal cortex and amygdala during acquisition and extinction of an active avoidance task. *Front. Behav. Neurosci* 9, 249 (2015). [PubMed: 26441578]
156. Lazaro-Munoz G, LeDoux JE & Cain CK Sidman instrumental avoidance initially depends on lateral and basal amygdala and is constrained by central amygdala-mediated Pavlovian processes. *Biol. Psychiatry* 67, 1120–1127 (2010). [PubMed: 20110085]
157. Martinez RCR et al. Active vs. reactive threat responding is associated with differential c-Fos expression in specific regions of amygdala and prefrontal cortex. *Learn. Mem* 20, 446–452 (2013). [PubMed: 23869027]
158. Ramirez F, Moscarello JM, LeDoux JE & Sears RM Active avoidance requires a serial basal amygdala to nucleus accumbens shell circuit. *J. Neurosci* 35, 3470–3477 (2015). [PubMed: 25716846]
159. Poremba A & Gabriel M Amygdalar Lesions Block Discriminative Avoidance Learning and Cingulothalamic Training-Induced Neuronal Plasticity in Rabbits. *J. Neurosci* 17, 5237 LP–5244 (1997). [PubMed: 9185561]
160. Moscarello JM & LeDoux JE Active avoidance learning requires prefrontal suppression of amygdala-mediated defensive reactions. *J. Neurosci* 33, 3815–3823 (2013). [PubMed: 23447593]
161. Kim EJ et al. Dynamic coding of predatory information between the prelimbic cortex and lateral amygdala in foraging rats. *Sci. Adv* 4, eaar7328 (2018). [PubMed: 29675471]
162. Burgos-Robles A et al. Amygdala inputs to prefrontal cortex guide behavior amid conflicting cues of reward and punishment. *Nat. Neurosci* 20, 824–835 (2017). [PubMed: 28436980]

163. Diehl MM, Iravedra-Garcia J, Gonzalez-Diaz F & Quirk GJ Interrogating inputs to rostral prelimbic cortex that drive active avoidance. in Molecular Cellular Cognition Society (2017).
164. Ramirez F, Moscarello JM, LeDoux JE & Sears RM Active Avoidance Requires a Serial Basal Amygdala to Nucleus Accumbens Shell Circuit. *J. Neurosci* 35, 3470–3477 (2015). [PubMed: 25716846]
165. Diehl MM, Iravedra-Garcia J, Gonzalez-Diaz F, Moran-Sierra J & Quirk GJ Interrogating the projections of rostral prelimbic cortex that drive active avoidance. in Society for Neuroscience Abstract No 502.07 (2018).
166. Diehl MM et al. Active avoidance requires inhibitory signaling in the rodent prelimbic prefrontal cortex. *Elife* 7, e34657 (2018). [PubMed: 29851381]
167. Baeg EH et al. Fast spiking and regular spiking neural correlates of fear conditioning in the medial prefrontal cortex of the rat. *Cereb. Cortex* 11, 441–451 (2001). [PubMed: 11313296]
168. Sotres-Bayon F, Sierra-Mercado D, Pardilla-Delgado E & Quirk GJ Gating of Fear in Prelimbic Cortex by Hippocampal and Amygdala Inputs. *Neuron* 76, 804–812 (2012). [PubMed: 23177964]
169. Martinez-Rivera FJ, Bravo-Rivera C, Velazquez-Diaz CD, Montesinos-Cartagena M & Quirk GJ Prefrontal circuits signaling active avoidance retrieval and extinction. *Psychopharmacology (Berl)*. 236, 399–406 (2019). [PubMed: 30259076]
170. Diehl MM, Bravo-Rivera C & Quirk GJ The study of active avoidance: a platform for discussion. *Neuro Biobehav Rev*
171. Stujenske JM, Likhtik E, Topiwala M. a. & Gordon J. a. Fear and Safety Engage Competing Patterns of Theta-Gamma Coupling in the Basolateral Amygdala. *Neuron* 83, 919–933 (2014). [PubMed: 25144877]
172. Choi J-S, Cain CK & LeDoux JE The role of amygdala nuclei in the expression of auditory signaled two-way active avoidance in rats. *Learn. Mem* 17, 139–147 (2010). [PubMed: 20189958]
173. Olton D & Isaacson R Hippocampal lesions and active avoidance. *Physiol. Behav* 3, 719–724 (1968).
174. Tonkiss J, Feldon J & Rawlins JNP Section of the descending columns of the fornix produces delay- and interference-dependent working memory deficits. *Behav. Brain Res* 36, 113–126 (1990). [PubMed: 2302311]
175. Weiner I, Feldon J, Tarrasch R, Hairston I & Joel D Finbria-fornix cut affects spontaneous activity, two-way avoidance and delayed non matching to sample, but not latent inhibition. *Behav. Brain Res* 96, 59–70 (1998). [PubMed: 9821543]
176. Gray J & McNaughton N Comparison between the behavioural effects of septal and hippocampal lesions: A review. *Neuro Biobehav Rev* 7, 119–188 (1983).
177. Schwarting R & Busse S Behavioral facilitation after hippocampal lesion: A review. *Behav. Brain Res* 317, 401–414 (2017). [PubMed: 27693851]
178. Wang J, Bast T, Wang Y-C & Zhang W-N Hippocampus and two-way active avoidance conditioning: Contrasting effects of cytotoxic lesion and temporary inactivation. *Hippocampus* 25, 1517–1531 (2015). [PubMed: 25926084]
179. Rosas-Vidal LE et al. Alteration of BDNF in the medial prefrontal cortex and the ventral hippocampus impairs extinction of avoidance. *Neuropsychopharmacology* 43, 2636–2644 (2018). [PubMed: 30127343]
180. Altemus M, Sarvaiya N & Neill Epperson C Sex differences in anxiety and depression clinical perspectives. *Front. Neuroendocrinol* 35, 320–330 (2014). [PubMed: 24887405]
181. Gamwell K et al. Fear Conditioned Responses and PTSD Symptoms in Children: Sex Differences in Fear-Related Symptoms. *Dev. Psychobiol* 57, 799–808 (2015). [PubMed: 26011240]
182. Lonsdorf TB et al. Sex differences in CS-discrimination during context-dependent fear learning and its retrieval - the role of biological sex, contraceptives and menstrual cycle phases. *J. Psychiatry Neurosci* 40, 368–375 (2015). [PubMed: 26107163]
183. Keiser AA et al. Sex Differences in Context Fear Generalization and Recruitment of Hippocampus and Amygdala during Retrieval. *Neuropsychopharmacology* 42, 397–407 (2017). [PubMed: 27577601]

184. Greiner EM, Müller I, Norris MR, Ng KH & Sangha S Sex differences in fear regulation and reward-seeking behaviors in a fear-safety-reward discrimination task. *Behav. Brain Res* 368, (2019).
185. Foilb AR, Bals J, Sarlito MC & Christianson JP Sex differences in fear discrimination do not manifest as differences in conditioned inhibition. *Learn. Mem* 25, 49–53 (2018). [PubMed: 29246981]
186. Day HLL, Reed MM & Stevenson CW Sex differences in discriminating between cues predicting threat and safety. *Neurobiol. Learn. Mem* 133, 196–203 (2016). [PubMed: 27423522]
187. Baratta MV et al. Behavioural and neural sequelae of stressor exposure are not modulated by controllability in females. *Eur. J. Neurosci* 47, 959–967 (2018). [PubMed: 29359831]
188. Shansky BRM Animal studies in both sexes. *Science* (80-.). 364, 825–826 (2019).
189. Kokras N, Hodes GE, Bangasser DA & Dalla C Sex differences in the HPA axis: an obstacle to antidepressant drug development? *Br. J. Pharmacol* 1–17 (2019). doi:10.1111/bph.14710
190. Diehl MM et al. Toward an integrative perspective on the neural mechanisms underlying persistent maladaptive behaviors. *Eur. J. Neurosci* 48, 1870–1883 (2018). [PubMed: 30044022]
191. Boeke EA, Moscarello JM, LeDoux JE, Phelps EA & Hartley CA Active Avoidance: Neural Mechanisms and Attenuation of Pavlovian Conditioned Responding. *J. Neurosci* 37, 4808–4818 (2017). [PubMed: 28408411]
192. Dunsmoor JE & Paz R Fear Generalization and Anxiety: Behavioral and Neural Mechanisms. *Biol. Psychiatry* 78, 336–343 (2015). [PubMed: 25981173]
193. Laufer O, Israeli D & Paz R Behavioral and Neural Mechanisms of Overgeneralization in Anxiety. *Curr. Biol* 26, 713–722 (2016). [PubMed: 26948881]

Highlights

- In this review, the authors provide an in-depth discussion of the behavioral processes and brain substrates within the amygdala-cortico-hippocampal circuit of:
- -learning safety through discrimination
- -learning safety through extinction
- -learning safety through active avoidance

Figure 1. Measuring behavioral responses of fear discrimination, generalization and safety.

A. Generalization levels can be tested after fear conditioning to a CS+ (orange) with an array of novel tone stimuli (a-e; black) of increasing similarity to the CS+. **B.** Generalization levels can also be assessed following fear conditioning to a cue or context predicting shock (CS+, CtxA+) and a separate cue or context predicting no shock (CS-, CtxB-); individuals show strong discrimination when the conditioned response is different (left graph), and strong generalization when the conditioned response is similar across cues/contexts (right graph). **C.** Schematic depicting procedural differences in experiments designed to investigate safety. In a within-subjects design (left) each subject is trained to both CS+ and CS- associations. In a between-subjects design (right) each subject is either trained to a CS+ or CS- association.

Figure 2: Proposed fear suppression circuitry engaged during an explicit safety cue, an extinguished fear cue, or avoidance signal.

Blue arrows indicate increased activity in projections associated with increased acquisition/consolidation of fear suppression. Green arrows indicate increased activity in projections onto local inhibitory interneurons associated with increased expression of learned fear suppression. Red arrows indicate projections where increased activity is associated with a loss of fear suppression.