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Review article

Know safety, no fear

Susan Sangha[a,*], Maria M. Diehl[b,*], Hadley C. Bergstrom[c,*], Michael R. Drew[d,*]

[a] Department of Psychological Sciences and Purdue Institute for Integrative Neuroscience, Purdue University, West Lafayette, IN, USA
[b] Department of Psychological Sciences, Kansas State University, Manhattan, KS, USA
[c] Department of Psychological Science, Program in Neuroscience and Behavior, Vassar College, Poughkeepsie, NY, USA
[d] Center for Learning and Memory and Department of Neuroscience, University of Texas at Austin, Austin, TX, USA

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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 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.

1.1. 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

* Corresponding authors.
E-mail addresses: sangha@purdue.edu (S. Sangha), maria.m.diehl@gmail.com (M.M. Diehl), habergstrom@vassar.edu (H.C. Bergstrom), mdrew@utexas.edu (M.R. Drew).

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(LeDoux, 2000; Pape and Pare, 2010)). 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 (Pape and Pare, 2010)), either directly or indirectly via a group of GABAergic intercalated cells (ITC) (Duvarci and Pare, 2014). 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 (Janak and Tye, 2015)). In addition to the BA, the LA also sends projections directly to the CeA to mediate conditioned fear responses (Li et al., 2013). The CeA is subdivided into lateral and medial zones based on connectivity, neurochemistry, and functionality (reviewed in (Pape and Pare, 2010)). The lateral division of the CeA (CeL) gates output from the medial division of the CeA (CeM) (Ciocchi et al., 2010), which projects to downstream hypothalamic and brainstem targets that orchestrate defensive responses (Wright and McDannald, 2019). 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 (Marek et al., 2013). 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 (Marek et al., 2018a). When the PL projections to the IL were optogenetically activated during fear extinction, faster extinction acquisition and better extinction recall was observed (Marek et al., 2018a). Bidirectional communication between amygdala and hippocampus has been shown to be important for establishing contextual representations (reviewed in (Maren and Holmes, 2016) and discussed further below). While much of the research on fear expression and suppression has been seated in the dorsal hippocampus (dHIPP) (reviewed in (Fanselow, 2010)), the amygdala and PFC do not receive direct dHIPP projections. Instead, the amygdala receives direct projections from ventral hippocampus (vHIPP) (Pitkanen, 2000), and the PFC receives direct projections from vHIPP and intermediate hippocampus (Cenquizca and Swanson, 2007; Jay and Witter, 1991).

2. Learning safety through discrimination

2.1. 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 (Dudai, 2002; Richards and Frankland, 2017). 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 (Shepard, 1987).

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 (Fig. 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 (Pollack et al., 2018). 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 (Fig. 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. (Sangha et al., 2013; Jovanovic et al., 2010)), 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 (Ostroff et al., 2010; Ronovsky et al., 2019)) (Fig. 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 (Pollak et al., 2008). 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 (Sangha et al., 2014a; Ng et al., 2018; Müller et al., 2018). 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.

2.2. Brain substrates

2.2.1. Amygdala

2.2.1.1. Lateral amygdala. In one influential model, the lateral amygdala (LA) is a key locus for the establishment of fear conditioning (reviewed in (LeDoux, 2000)). 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 (Ostroff et al., 2010; Ghosh and Chattarji, 2015; Grosso et al., 2018; Rogan et al., 2005). 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) tone (Ghosh and Chattarji, 2015). 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 stimuli (Grosso et al., 2018). 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 CS (Grosso et al., 2018). 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 al. (Ostroff et al., 2010) 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 al. (Rogan

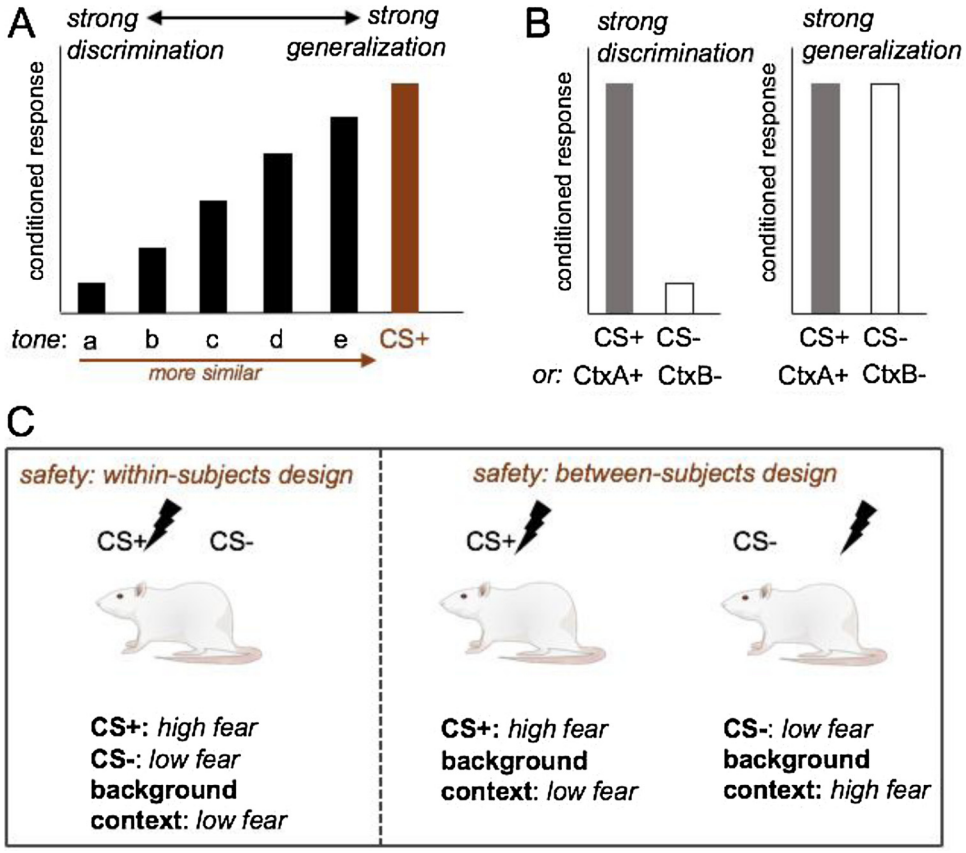

Fig. 1. Measuring behavioral responses of fear discrimination, generalization and safety. A. Generalization levels can be tested after fear conditioning to a CS + (orange) with an array of novel tone stimuli (a–e; black) of increasing similarity to the CS + . B. Generalization levels can also be assessed 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.

et al., 2005) 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.

2.2.1.2. Basal amygdala. The BA is often described as an integration hub within the amygdala (reviewed in (Janak and Tye, 2015)). 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 (Sangha et al., 2013). 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 (Christianson et al., 2012). Many of the BA neurons that responded to the safety and not the fear cues, became responsive to the fear cue following fear extinction (Sangha, 2015). 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 (Janak and Tye, 2015)) has been recently challenged. When Kyriazi et al. (Kyriazi et al., 2018) 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.

2.2.1.3. 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 (Ciocchi et al., 2010; Botta et al., 2015; Sanford et al., 2017; De Bundel et al., 2016). In a pioneering series of experiments, Ciocchi et al. (Ciocchi et al., 2010) 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^+) (Haubensak et al., 2010). Supporting a role of CeL PKC-δ^+ "off" cells in fear generalization, photostimulation of these cells resulted in greater

responding to a CS- (generalization) (Botta et al., 2015). In further support of a role for CeA GABAergic PKCδ$^+$ "off" cells in fear memory generalization, α5-GABA receptor-mediated extra-synaptic inhibition knock-down specifically on PKCδ$^+$ cells in the CeA resulted in enhanced fear memory generalization (Botta et al., 2015). These data support a modulatory role for extra-synaptic α5-GABA receptors in PKCδ$^+$ cells in the CeA and fear memory generalization.

In addition to the discussed role for PKCδ$^+$ cells in fear memory generalization, another distinct population of cells in the CeA are selective for fear discrimination (Sanford et al., 2017). 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 discrimination (De Bundel et al., 2016). Blockade of DA D2 receptors in the CeA increased generalization, while activation of DA terminals in the CeA reversed memory generalization (De Bundel et al., 2016). 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 (Sanford et al., 2017; Shaban et al., 2006; Bergado-Acosta et al., 2008; Lange et al., 2014; Sangha et al., 2009), 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 (PKCδ$^+$) 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.

2.2.2. 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 (Herry et al., 2008; Milad and Quirk, 2012; Giustino and Maren, 2015), as well as stimulus discrimination and generalization (Pollack et al., 2018; Grosso et al., 2018; Vieira et al., 2014, 2015; Scarlata et al., 2019; Sangha et al., 2014b). 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 generalization (Vieira et al., 2014). 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- (Vieira et al., 2015). 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 (Milad and Quirk, 2012; Giustino and Maren, 2015), with the IL implicated in fear suppression and PL implicated in fear expression (Sierra-Mercado et al., 2011). 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) (Pollack et al., 2018). 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 (Grosso et al., 2018). Recently, it was shown that chronic alcohol magnifies fear memory generalization and overgeneralization was associated with reduced expression of Arc in the IL (Scarlata et al., 2019). Chemogenetic stimulation of the IL completely reversed the effects of alcohol on fear memory generalization, implicating activity in the IL with discrimination (fear suppression) (Scarlata et al., 2019).

Dissociable roles of the PL and IL have also been observed during discrimination among fear, safety and reward cues (Sangha et al., 2014b). 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. (Corches et al., 2019) 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 (Corches et al., 2019). Overall, an accumulation of evidence points to modulatory, and potentially dissociable roles, for the PL and IL in fear generalization and discrimination.

2.2.3. 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 (Huckleberry et al., 2016; Rudy, 2009). Perturbations to both dorsal and ventral hippocampus impair acquisition and expression of such context-evoked fear (Matus-Amat et al., 2004; Rudy and Matus-Amat, 2005; Huckleberry et al., 2018) (reviewed in (Fanselow, 2010)). Because excessive generalization of learned contextual fear is believed to contribute to anxiety and stress-related disorders (Bouton et al., 2001; Kheirbek et al., 2012; Mineka and Zinbarg, 2006; Sahay et al., 2011a; Barlow, 2002), 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 (O'Reilly and McClelland, 1994; Treves and Rolls, 1992). 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 (Leutgeb et al., 2007). Furthermore, when rodents are exposed to several different environments, DG granule cells typically fire in only a single environment (GoodSmith et al., 2017), 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 (Kesner, 2018; Rolls and Kesner, 2006). 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 other (Gilbert et al., 2001). Perturbations of DG also impair specificity of contextual fear memory. McHugh et al. (McHugh et al. (2007)) 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 (Moreno-Jiménez et al., 2019; Eriksson et al., 1998; Kempermann et al., 2018). 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 (Huckleberry et al., 2018; Saxe et al., 2006; Drew et al., 2010), as well as context fear discrimination in mice (Tronel et al., 2012; Niibori et al., 2012). Moreover, artificially boosting neurogenesis by preventing apoptosis of newborn neurons improves context fear discrimination (Sahay et al., 2011b). The circuit mechanisms for these effects are still unclear, but there is some evidence that neurogenesis promotes sparse coding of contexts within DG (Niibori et al., 2012; Ikrar et al., 2013), thereby supporting the pattern separation function of DG (reviewed in (Drew and Huckleberry, 2017)).

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. (Bernier et al. (2017)) 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 (Fanselow and Dong, 2010; Moser and Moser, 1998). 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 (Marek et al., 2018b; Xu et al., 2016). 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 (Matus-Amat et al., 2004; Rudy and Matus-Amat, 2005). Based on evidence that dorsal hippocampus generates more precise spatial representations than ventral hippocampus (Royer et al., 2010; Ruediger et al., 2012), 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 cortex (Amaral and Witter, 1989).

2.3. 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 regulated (Xu and Südhof, 2013), 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 linking (Cai et al., 2016) 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

3.1. Behavioral processes

Extinction was first described by Pavlov (Pavlov (1927)), 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 (Bouton et al., 2001;

Mineka and Zinbarg, 2006). Deficits in fear extinction have been hypothesized to contribute to disorders such as PTSD (Jovanovic et al., 2010; Lissek and van Meurs, 2015).

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 recovery (Pavlov, 1927). 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 reinstatement (Lissek and van Meurs, 2015), meant that extinction was not caused by simple fatigue of the sensory or motor systems. Bouton and colleagues (Bouton, 2004) 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. (Maren and Holmes, 2016; Marek et al., 2018b; Lingawi et al., 2018; Singewald and Holmes, 2019; Goode and Maren, 2019)). Here we will provide a brief summary, highlighting findings within the amygdalo-cortico-hippocampal circuit.

3.2. Brain substrates

3.2.1. Amygdala

Acquisition of fear extinction requires NR2B-containing NMDA receptors in the lateral amygdala (Sotres-Bayon et al., 2007). However, extinction can also induce depotentiation at LA synapses that were potentiated after fear conditioning (Kim et al., 2007), 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 colleagues (Herry et al., 2008) 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 extinction (Likhtik et al., 2008) and provide increased inhibition onto CeA fear output neurons after extinction (Amano et al., 2010). 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 decarboxylase (Sangha et al., 2012). 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.

3.2.2. 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 itself (Milad and Quirk, 2002). 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 (Milad and Quirk, 2002; Vidal-gonzalez et al., 2006). Conversely, when the IL was pharmacologically inactivated during extinction acquisition, the extinction acquisition curve was negatively affected and recall the next day was impaired (Sierra-Mercado et al., 2011). Interestingly, when IL inactivation was limited to the consolidation phase during relearning of extinction, poor memory for extinction was observed during recall (Laurent and Westbrook, 2009). 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 (Sangha et al., 2014b; Laurent and Westbrook, 2009) (but see (Do-Monte et al., 2015)), 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 recall (Lesting et al., 2011). 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 extinction (Senn et al., 2014). When the IL input to the BLA (LA + BA combined) was optogenetically increased or decreased during extinction acquisition, Bukalo et al. (Bukalo et al., 2015) 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- (Likhtik et al., 2014). 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 reduced (Sierra-Mercado et al., 2011). 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-free (Laurent and Westbrook, 2009). 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 training (Milad and Quirk, 2002). Furthermore, PL recordings made during a discriminative fear conditioning task found that PL neurons were activated during both conditioned and generalized stimuli (Corches et al., 2019), 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 seen (Giustino et al., 2016).

3.2.3. 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 not (Bouton et al., 2006). 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 al. (Marek et al., 2018c) 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 day (Sierra-Mercado et al., 2011).

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 place (Kim and Fanselow, 1992). Such contextual fear conditioning (CFC) requires plasticity in the hippocampus, which is necessary for generating a conjunctive representation of the context (Fanselow, 2000; O'Reilly and Rudy, 2001) that binds together multiple features from different sensory modalities (Huckleberry et al., 2016). Like tone fear extinction, context fear extinction requires integrity of the mPFC (Laurent and Westbrook, 2009). 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 amygdala (Rudy, 2009). 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 processes (Fanselow, 1990), that rodents readily fear condition to remembered context representations (Rudy et al., 2002), and that fear conditioning can be induced by pairing artificial reactivation of a hippocampal context representation with shock (Ramirez et al., 2013).

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 al. (Moita et al., 2003) 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. (Wang et al. (2012)) 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 group (Wang et al., 2015a) 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 memory (Bernier et al., 2017). Tronson et al. (Tronson et al., 2009) 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. (Lacagnina et al., 2019) 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. (Khalaf et al. (2018)) 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. (Lacagnina et al. (2019)) and Khalaf et al. (Khalaf et al. (2018)) 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 acquisition (Drew and Brockway, 2019). 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 (Anagnostaras et al., 1999; Corcoran et al., 2013), 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. (Song et al. (2010)) 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 (Ledoux et al., 1989; Falls and Davis, 1993). Olfactory fear conditioning potentiates the synaptic output of olfactory sensory neurons (Kass et al., 2013), 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.

3.3. 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 fear (An et al., 2017). Similarly, behavioral and pharmacological interventions such as retrieval-extinction (Monfils et al., 2009), immediate extinction (Myers et al., 2006; Bernier et al., 2014), pharmacological blockade of reconsolidation (Kindt et al., 2009; Nader et al., 2000), and epigenetic manipulations (Stafford et al., 2012) may more effectively suppress fear than traditional extinction training. However, many questions remain about the generality, effectiveness, and mechanisms of these procedures (for review see (Maren, 2011)). 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

4.1. 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 (Bolles et al., 1966; Gabriel, 1968), lever press avoidance (D'Amato and Schiff, 1964; Berger and RF, 1975; Servatius et al., 2008), and shuttle avoidance (Mowrer, 1939; Kamin et al., 1963; Mowrer, 1960; Bolles, 1971).

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 (Mowrer, 1939; Kamin et al., 1963; Mowrer, 1960). 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 (Mowrer and Lamoreaux, 1946; Levis, 1989). 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 (LeDoux et al., 2017; Dinsmoor, 2001; Cain, 2019; Bolles, 1970); 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 (LeDoux et al., 2017; Cain, 2019).

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 platform (Bravo-Rivera et al., 2014). 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 pre-clinical model because patients excessively avoid at the cost of goal-directed behaviors (reviewed in this issue (Diehl et al., 2019)).

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 predator (Choi and Kim, 2010). 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.

4.2. Brain substrates

4.2.1. 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 avoidance (Jiao et al., 2015) and is necessary for acquiring shuttle (Choi and Kim, 2010; Lazaro-Munoz et al., 2010; Martinez et al., 2013; Ramirez et al., 2015a) and wheel-running avoidance (Poremba and Gabriel, 1997). Following acquisition of platform-mediated avoidance, pharmacological inactivations of the BLA have blocked avoidance expression (Bravo-Rivera et al., 2014). Interestingly, cFos and lesion studies support the need for prefrontal suppression of the central amygdala in allowing the shuttle response to occur (Choi and Kim, 2010; Moscarello and LeDoux, 2013). 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 colleagues (Choi and Kim, 2010), 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) (Kim et al., 2018). 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 cues (Burgos-Robles et al., 2017). 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 photo-exciting 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 avoidance (Diehl et al., 2017). 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 response (Ramirez et al., 2015b). Consistent with these findings, preliminary results using the platform-mediated avoidance task show that photosilencing BLA projections to VS impaired active avoidance (Diehl et al., 2018a). Together, these findings support that amygdala inputs to the VS are necessary for the expression of active avoidance.

4.2.2. 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 avoidance (Martinez et al., 2013), and IL lesions impair its expression (Moscarello and LeDoux, 2013). Studies using lever press avoidance have also shown activation of both PL and IL during the expression of active avoidance (Jiao et al., 2015). Furthermore, platform-mediated avoidance was disrupted following PL inactivation (Bravo-Rivera et al., 2014; Diehl et al., 2018b), but IL inactivation had no effect (Bravo-Rivera et al., 2014). 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 conditioning (Diehl et al., 2018b). Increased PL activity was correlated with freezing following fear conditioning (Burgos-Robles et al., 2017; Baeg et al., 2001; Sotres-Bayon et al., 2012), but decreased activity at tone onset was only observed in avoidance-trained rats, suggesting that PL inhibition is correlated with avoidability (Diehl et al., 2018b). 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 BLA (Martinez-Rivera et al., 2019). Ongoing studies are currently addressing which targets of PL are necessary for avoidance using an optogenetic approach (Diehl et al., 2018a). 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 issue (Diehl, 2019))

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 (Likhtik et al., 2014; Stujenske et al., 2014), and that activating PL-BLA projections drive active avoidance (Diehl et al., 2018a; Martinez-Rivera et al., 2019). 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 (Moscarello and LeDoux, 2013; Choi et al., 2010) (reviewed in (LeDoux et al., 2017; Cain, 2019)).

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 danger (Kyriazi et al., 2018). 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.

4.2.3. Hippocampus

Early studies have shown that permanent hippocampal lesions facilitate shuttle avoidance (Olton and Isaacson, 1968; Tonkiss et al., 1990; Weiner et al., 1998) (reviewed in (Gray and McNaughton, 1983; Schwarting and Busse, 2017)), whereas transient pharmacological inactivations of either dorsal or ventral hippocampus impair the expression of shuttle avoidance (Wang et al., 2015b). 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 response (Rosas-Vidal et al., 2018). Similar to the role of the ventral hippocampus in the extinction of fear (Sierra-Mercado et al., 2011; Sotres-Bayon et al., 2012), the ventral hippocampus likely modulates the expression of platform avoidance by mediating behavioral flexibility.

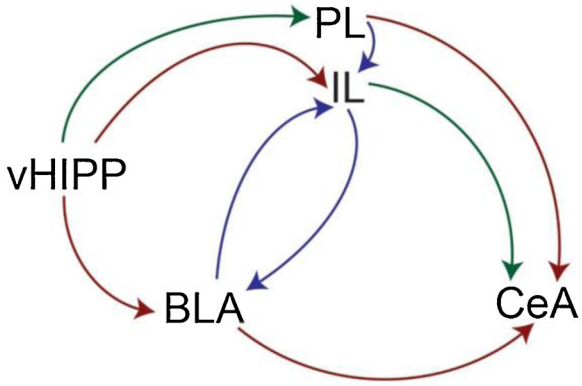

Fig. 2. Proposed fear suppression circuitry engaged during an explicit safety cue, an extinguished fear cue, or avoidance signal. Blue arrows indicate increased activity in projections associated with increased acquisition/consolidation of fear suppression. Green arrows indicate increased activity in 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.

4.3. 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 disorder (Altemus et al., 2014). 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 (Gamwell et al., 2015; Lonsdorf et al., 2015). 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 observed (Keiser et al., 2017). In a paradigm where the animal needed to discriminate among fear, safety and reward cues, Greiner et al. (Greiner et al., 2019) 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 al. (Foilb et al., 2018) 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 training (Day et al., 2016). 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 freezing (Baratta et al., 2018). 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 females (Baratta et al., 2018). Interestingly, omission of a safety signal facilitated extinction of avoidance in females, but not males (Baratta et al., 2018). 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 (Shansky, 2019; Kokras et al., 2019).

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 (Fig. 2). Additionally, increased activity in PL neurons projecting to the IL enhances acquisition of fear extinction (Marek et al., 2018a). 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 suppression (Sotres-Bayon et al., 2012). 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 disorders (Diehl et al., 2018c). 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 threat (Boeke et al., 2017). 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) (Dunsmoor and Paz, 2015; Laufer et al., 2016). 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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