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Prefrontal cortical regulation of fear learning

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Abstract

The prefrontal cortex regulates the expression of fear based on previously learned information. Recently, this brain area has emerged as critical in the initial formation of fear memories, providing new avenues to study the neurobiology underlying aberrant learning in anxiety disorders. Here we review the circumstances under which prefrontal cortex is recruited in the formation of memory, highlighting relevant work in both laboratory animals and human subjects. We propose that prefrontal cortex facilitates fear memory through integration of sensory and emotional signals and through coordination of memory storage in an amygdala-based network.

Keywords

fear conditioning; prelimbic; memory; learning; PFC; fMRI; awareness

An expanded role for prefrontal cortex in fear memory

Adaptive responding to threat is critical for survival. Learning to avoid cues that predict danger and approach cues that predict safety depends on highly conserved neural circuitry. Anxiety disorders manifest when threat assessment becomes maladaptive, leading to exaggerated physiological and behavioral reactions to perceived or anticipated threat or inappropriate fear to non-threatening situations. Because roughly 18% of the U.S. population may suffer from an anxiety disorder [1], understanding the neurobiology of fear and anxiety is an important goal. At its core, threat assessment requires accurately predicting an aversive outcome from available environmental signals. For this reason, fear conditioning has proved to be a powerful tool to investigate the neurobiology supporting emotional learning and fear expression in both human and non-human subjects. Fear conditioning studies have described a critical role for the amygdala, hippocampus, and cerebral cortex in

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the regulation of fear memory and behavioral expression. Standard fear conditioning requires subjects to associate a neutral conditional stimulus (CS) such as a tone with an aversive unconditional stimulus (UCS) such as a shock. After repeated CS-UCS pairings, the CS elicits conditional fear responses (CRs) in the subject, including changes in autonomic activity, analgesia, and freezing behavior. The power of this procedure comes from its simplicity, rapid acquisition, sensitivity to cellular/molecular-, genetic-, and systems-level manipulations, and its translational application. Early work characterizing the circuitry for fear conditioning identified the amygdala as a critical site for memory formation and CS-UCS convergence during learning [2, 3]. For the past 25 years, the use of fear conditioning has greatly advanced our understanding of memory formation, consolidation, and stability with a primary focus on the amygdala [2, 4]. Likewise, more complex variants of the procedure, such as contextual fear conditioning and trace fear conditioning, have been used to study hippocampal and cortical contributions to emotional memory. The prefrontal cortex (PFC) has attracted substantial interest in recent years for its ability to bidirectionally modulate the expression of previously learned fear [5, 6]. Ventral PFC in the rat appears to be necessary for controlling fear to a CS that no longer predicts danger, as in extinction learning [7, 8]. In contrast, dorsal PFC was found to promote the expression of learned fear. Similar complementary patterns of activation have been observed in human dorsal and ventral PFC subregions, suggesting possible top-down regulation of amygdala circuitry in adaptive responding to threat [9].

In addition to regulating the behavioral expression of existing fear memories, it is becoming clear that prefrontal neurons are engaged in various aspects of fear memory formation. An appreciation of how the PFC might regulate the initial formation of aversive memories is critical to determining how dysfunction in cortical-subcortical circuits leads to maladaptive threat assessment in anxiety disorders. Here we review the circumstances under which the PFC appears necessary for fear memory based on evidence from work in laboratory animals and humans. We discuss the functional and anatomical heterogeneity of the PFC as it relates to fear memory regulation and point to avenues of future study that ultimately will improve our understanding of emotional memory formation.

Prefrontal cortical recruitment in fear memory

Fear expression vs. fear learning

Prefrontal cortex was initially implicated in emotional regulation based on reports of emotional and behavioral dysregulation after prefrontal damage [10]. This prompted a closer examination of the role of this structure in emotional learning using a standard fear conditioning paradigm (also known as “delay” fear conditioning, DFC), in which the UCS is delivered at the end of a discrete stimulus like a light or a tone. However, early lesion studies found that the PFC was not required for learning the basic CS-UCS association, but instead might participate in the extinction of cued fear [8]. This general pattern has been observed repeatedly using lesions and temporary inactivation [11–13], leading to the generally accepted conclusion that the PFC contributes to the regulation of previously learned fear rather than to forming the initial CS-UCS association. The initial learning of the association may instead be supported largely by the amygdala and plasticity in sensory

systems. Sensory information from the CS and UCS converges in the lateral nucleus of the amygdala via thalamus [3], and subsequent processing through amygdala connections with the hypothalamus and brainstem nuclei produces conditional fear responses to the CS [reviewed in 2]. This subcortical fear circuit allows rapid automatic responding to threatening stimuli without the need for cortical processing.

The recruitment of the PFC to regulate fear expression based on previously learned information fits with known roles of this region in cognitive control and flexibility, i.e., coordinating action through integration of diverse mnemonic inputs and top-down regulation of specific brain circuits [14]. Contextual control of extinction, for example, requires input from the hippocampus to PFC for the appropriate expression of fear responses in a new context, but not in the extinction-related, or safe, context [15]. These higher-order cognitive functions are not necessary for the basic association of the CS and UCS. However, work in the past decade has revealed that the PFC also contributes to the initial learning of fear in more cognitively demanding variants of fear conditioning. For example, the insertion of an empty temporal gap, or “trace interval”, between the CS and UCS renders learning the association critically dependent on the PFC [11, 16–19]. In some cases, the association of complex contextual stimuli with shock also requires the PFC [11, 18, 20]. It is possible that the added spatial and temporal complexity in these training procedures may require cognitive functions attributed to the PFC, particularly working memory, attention, and shock expectancy or contingency evaluation. Furthermore, the examination of the PFC in memory formation is beginning to reveal that even in standard delay conditioning PFC may normally regulate the formation of the association [18, 21, 22]. This is not surprising given that in both humans and laboratory animals PFC subregions exhibit changes in learning-related activity during delay conditioning [23–27]. In general, associative fear requires plasticity in a distributed network of brain structures [4] and the PFC may contribute to memory *formation* in this network in addition to regulating the subsequent *expression* of fear. In the next section we discuss findings from trace and contextual fear conditioning, which provide an avenue for studying the role of the PFC in fear memory formation.

Trace and contextual fear conditioning

Dorsal regions of the PFC are necessary for associative fear learning when temporal or contextual complexity is introduced. In trace fear conditioning, a cue predicts the occurrence of an aversive shock that will occur many seconds later. The association of the cue and shock cannot be supported by simultaneous sensory stimulation converging on amygdala neurons as can be the case for delay conditioning. Thus, additional circuitry is recruited to process this temporal component, including the prefrontal cortex, hippocampus, and entorhinal and perirhinal cortices [11, 17, 28–33]. The precise role of each structure is largely unknown, but it is thought that activity in one or more of these structures may support trace conditioning by providing a bridging signal between representations of the CS and UCS. While some computational models suggest that the hippocampus might provide a bridging signal [34, 35], neither CA1 nor DG areas exhibit firing patterns consistent with providing this signal [36]. More recently, the PFC has emerged as a strong candidate for this function. Cue-initiated persistent firing lasting several seconds had been well documented in studies of working memory in primates. Recording studies in trace fear conditioning showed

that units in PFC maintain firing past CS-offset, into the trace interval, for both short 2-sec [24] and long 20-sec intervals [37] (Figure 1A). These “bridging” cells are observed in the dorsal, prelimbic (PL) area, but not the ventral, infralimbic (IL) area [37]. Similar results have been obtained in rabbits performing trace eyeblink conditioning with persistent firing neurons located primarily in deep, output layers of dorsal PL and anterior cingulate cortex (ACC) [38–40]. This anatomical position is in line with a model where PL provides a bridging signal allowing CS-activated networks to coincide with UCS delivery. Elegant work out of the Mauk lab has provided physiological support for such a model. Electrical stimulation of cortical input to cerebellum during the CS and trace interval was sufficient to support acquisition of eyeblink CRs in the absence of a functioning PFC [41]. Additional lines of evidence provide indirect support for a bridging role for the PFC in associative fear learning. Molecular mechanisms important for persistent firing of cortical cells, such as activation of NR2B-containing NMDA receptors and muscarinic acetylcholinergic (mACh) receptors, are important for trace fear conditioning [18, 42]. We recently directly tested the requirement of prefrontal trace interval bridging activity to learning using optogenetic silencing of prelimbic neurons during the trace interval [19]. Silencing PL activity during the 20-sec trace interval, but not during the CS or inter-trial interval, prevented the development of fear to the CS (Figure 1). This finding showed for the first time that prefrontal cortical activity is likely to link discrete events in memory. The next challenge is to determine the information content of this bridging activity. It is unlikely to be sensory processing per se, a function that may be supported by persistent firing in perirhinal cortex [30, 43]. Instead, it may reflect the maintenance of attentional resources during the CS-UCS interval and/or the coordination of associative encoding downstream in amygdala and rhinal cortices. Whether this activity contributes to local storage of the association in the PFC is also a question of current interest (Box 1).

A specific role for prefrontal cortex in contextual learning is less clear. Contextual fear conditioning is largely supported by the hippocampus and amygdala. Lesions or inactivation of PFC typically leave contextual fear memory intact if the context is the sole predictor of the shock (i.e., “foreground” contextual learning), as in shock-only training, unpaired pseudoconditioning, or differential contextual conditioning [11, 12, 44–46]. However, the PFC appears to be necessary for contextual fear learning if the context itself is not the only reliable predictor of shock (i.e., “background” contextual learning). Inactivation of either PL or ACC prior to auditory cued fear conditioning impairs memory for the training context [11, 20]. Lesions of more ventral PFC can actually enhance contextual fear memory [47]. Thus, the PFC may regulate memory formation based on the relative predictive value of the available stimuli. Consistent with this idea, the lateral amygdala might also participate differentially in foreground and background contextual conditioning since one study [48] showed that lesions of lateral amygdala impair foreground, but enhance background context learning. The authors suggested that in the presence of a discrete predictor of the UCS, lateral amygdala may help to suppress learning to the background context. The PFC may assess the predictive value of multiple excitatory cues and regulate activity in the amygdala during learning. These findings from trace and contextual fear conditioning point to a working model of prefrontal regulation of fear learning in which PFC integrates information

about the temporal, contextual, and predictive relationship of the cue and shock in order to influence memory storage in amygdala circuitry (Box 2).

Neurobiological mechanisms of prefrontal function in emotional learning

The circumstances under which the PFC regulates fear learning reflects features of higher-order cognitive function, such as working memory, attention, and top-down regulation of subcortical neural systems. In this way trace and contextual fear conditioning studies in laboratory animals bring animal work further in line with findings in human subjects. For example, acquisition of trace, but not delay, conditioning in humans requires conscious awareness [49–51]. This requirement is correlated with selective activation of hippocampus and frontal cortical regions in trace conditioning (Figure 2) [27, 52, 53]. Activation of dorsal PFC regions in general during emotional situations is associated with conscious appraisal of threat [54, 55], and activation of dorsolateral PFC and dorsal ACC are thought to represent working memory and attentional processes, respectively [52, 56, 57]. Trace and contextual fear conditioning studies in animals may therefore provide some insight into the underlying neurobiology of these complex cognitive concepts.

Working memory

Working memory-like processes during fear learning, as mentioned above, require persistent activity during the CS-UCS interval [19, 37], which may depend in part on glutamatergic signaling mediated by NR2B-containing NMDA receptors [18, 58]. NMDA receptor mediated signaling is critical for trace fear conditioning as well as contextual fear learning [11, 18]. Importantly, prefrontal NMDARs containing the NR2B subunit have a selective role in the trace fear association compared with the contextual association [18]. NR2B-containing subunits confer a longer deactivation window to NMDA-mediated current, a feature which is amenable to persistent firing in recurrent networks [59, 60]. NR2B has recently been shown to be necessary for working memory [58, 61]. Thus, the selective involvement of NR2B-containing NMDARs in trace but not contextual fear lends support for a working memory function of prefrontal persistent firing in trace conditioning. Interestingly, a similar specificity of NR2B-mediated signaling for trace but not contextual fear conditioning has been observed in the hippocampus [62]. Although hippocampal neurons do not exhibit persistent firing during trace fear conditioning, NR2B-mediated signaling nonetheless supports temporal processing in the hippocampus. Selective knockout of NR2B-containing NMDA receptors in CA1 impairs spatial working memory but leaves Morris water maze performance intact [63]. Moreover, hippocampus and PFC are co-activated during trace fear conditioning in humans and rodents, and communication between these two structures is important for spatial working memory [27, 36, 37, 64, 65]. Further examination of the cellular signaling downstream of these receptors will likely provide insight into how hippocampus and PFC contribute to contextual versus temporal/episodic regulation of emotional memory.

Cholinergic signaling mediated by muscarinic acetylcholine receptors (mAChR) may also contribute to successful encoding of fear memory in PFC. Systemic delivery of the mAChR antagonist scopolamine impairs performance in both trace fear conditioning and working memory [42, 66] as well as persistent firing in the dlPFC of primates [66]. Facilitating

cholinergic signaling with physostigmine enhances trace fear conditioning [67]. Cholinergic activation via muscarinic AChR has been shown to be necessary for persistent firing and trace conditioning in several other cortical regions [31, 43, 68], suggesting a common cortical mechanism in working memory or transient bridging activity supporting emotional memory formation.

Attention

Prefrontal activity during trace conditioning may also reflect the regulation of attentional resources during cue-shock pairings. Trace conditioning, to a much greater extent than delay conditioning, is impaired if human subjects are given a distracting task during the period of CS-UCS pairings [69]. Similarly, in mice, distracting visual stimuli impair trace, but not delay fear conditioning [45]. Han and colleagues provide indirect support for ACC mediating this effect. Trace conditioning led to greater c-fos expression in ACC compared with delay and foreground contextual conditioning [45], and pre-training lesions of ACC with NMDA selectively impaired trace conditioning [45]. Pre-training intra-ACC injection of NMDAR NR2B antagonists also impairs trace fear conditioning [70]. Whether these receptors in ACC serve a role in attention distinct from that in persistent firing and working memory remains to be determined. Dorsal PL/ACC of the rodent shares functional and anatomical homology with primate ACC in attention regulation [57, 71–73]. Attention regulation may underlie a common function of the PFC in emotional learning in general. Recent work has implicated PL and ACC, but not IL, in detection of prediction error and regulation of attention based on UCS expectancy [25, 74, 75]. The element of surprise from an unexpected outcome is a fundamental requirement for learning in several learning models [76] and expression of c-fos in PL increases specifically when the UCS is unexpected early in training [25]. After sufficient training, the CS is a reliable predictor of the UCS, and if a novel cue is presented in compound with this well-learned CS, associating the new cue with the shock is impaired, a phenomenon called blocking. Interestingly, inactivating PL during this compound training unblocks fear learning to the new CS [25]. This suggests that the PL is regulating attentional resources to this new cue based on associative information from other available cues. In support, Buchel and colleagues [77] used eye-tracking to show that human subjects tracked the more predictive stimulus for shock of the compound stimulus during a blocking paradigm. Furthermore, gaze fixation time correlated with BOLD responses in both the ACC and amygdala, suggesting a possible functional interaction between these two regions in attending to predictive cues.

Reconsidering a role for prefrontal cortex in delay fear conditioning

While more complex variants of basic fear conditioning, such as trace and contextual fear learning, critically depend on prefrontal cortical function, expression of fear in delay conditioning can clearly occur in the absence of the PFC. In humans, conscious awareness is not required for the association of the CS and UCS when they co-terminate [49, 50]. Nonetheless, some prefrontal cortical regions, such as middle frontal gyrus and ACC, do exhibit changes in activity in delay fear conditioning and may reflect explicit processing of fear cues or their predictive value [23, 26, 27, 78]. Similarly, in rodents, neurons in PFC are active during delay fear conditioning [24, 25, 79] and these responses can be modulated by

amygdala input [22, 79, 80]. These activation patterns may reflect a role for the PFC in mediating the expression of fear during learning, or they may reflect the appraisal of the predictive value or the salience of fear cues [12, 25, 54]. Dissecting these possibilities in delay conditioning has been difficult because lesions or temporary inactivation do not normally prevent learning [8, 11, 12], but more selective manipulations of specific molecular pathways or monoamine signaling are beginning to reveal that the PFC normally contributes to delay fear learning. Disruption of dopamine signaling in PFC impairs olfactory delay conditioning in addition to auditory trace conditioning and fear extinction [22, 81, 82]. Dopamine in prefrontal networks may regulate input from other regions [83, 84] and disrupting dopaminergic tone could thus affect cue salience or the integration of subcortical input during memory formation. In addition to dopaminergic signaling, cannabinoid signaling is important for normal delay fear conditioning. Signaling via the CB1 receptor in PFC is necessary for olfactory conditioning and activation of the CB1 receptor potentiates unit responses in PFC to fear cues as well as facilitates memory retention [85]. Importantly, PFC's role in delay fear conditioning involves bi-directional communication with the amygdala [22, 80, 85–87].

Fear learning and prefrontal network balance

A possible explanation for these discrepant findings in prefrontal regulation of delay conditioning comes from recent work by Yizhar and colleagues using optogenetics to probe network dynamics in the PFC. Elevation of the excitatory/inhibitory (E/I) balance in prefrontal networks by selectively increasing excitatory tone impaired delay fear conditioning to an auditory cue [21]. Decreasing E/I balance by increasing inhibitory tone or inactivating excitatory cells with halorhodopsin did not affect learning. These results suggest that manipulations that selectively reduce inhibitory tone may be the important factor in prefrontal regulation of memory formation in delay fear conditioning. Disruption in E/I balance within prefrontal networks via aberrant activity of parvalbumin (PV)-containing inhibitory cells has been hypothesized to underlie cognitive dysfunction in schizophrenia and autism [88, 89] and may underlie dysregulated emotional memory in these disorders. A recent study by Herry and colleagues showed that PV-containing interneurons are necessary for the expression of learned fear through regulation of principal neuron firing [90]. Previous work has demonstrated a correlation between prefrontal firing and the expression of fear behavior [91] and disinhibition of principal cell firing by PV-containing interneurons promotes fear expression [90]. While these studies appear to suggest that PV-regulation of cell firing may have opposing effects on fear learning and fear expression, they clearly highlight the importance of network balance in emotional memory. The manipulations of dopaminergic, glutamatergic, and cannabinoid signaling previously described which impair delay fear conditioning may primarily affect inhibitory tone in the PFC. Dopaminergic input from the ventral tegmental area acting on D2 and D4 receptors has been shown to regulate the balance of excitatory and inhibitory transmission in the BLA-PFC circuit [92]. CB1 receptors in the PFC are located on inhibitory terminals and may modulate local inhibition [93, 94]. Recently, we showed that a NR2A-preferring antagonist impaired the acquisition of trace, contextual, and delay fear conditioning [18]. PV-containing interneurons express proportionally more NR2A-containing NMDARs compared with principal cells, [95], and NR2A antagonists may disrupt glutamatergic activation of inhibitory networks in the PFC.

Another recent study found that the perineuronal net in the PL PFC is necessary for cued fear including both trace and delay conditioning [96]. The perineuronal net is the extracellular matrix surrounding neurons, which is thought to regulate synaptic stabilization during learning. In the PFC, this net is more intense around PV-positive inhibitory neurons [96]. Interestingly, the amygdala, which is required for the reported effects of cannabinoid and dopamine signaling on fear learning [22, 80], typically suppresses prefrontal firing, possibly via glutamatergic action on PV interneurons [79, 92, 97]. Taken together, these findings contribute to an emerging picture that amygdala regulation of prefrontal network activity is necessary for the adaptive encoding of cued fear. This opens up the possibility that genetic disruption in these prefrontal circuits contributes to aberrant threat assessment in the development of anxiety disorders.

Concluding remarks

This review has highlighted an expanded role for the PFC in the regulation of fear memory. Not only is the PFC important for regulating the behavioral expression of fear and extinction of previously acquired fear memories, but the PFC is also critical for the initial formation of emotional memories involving sufficient temporal or contextual complexity. This broadened understanding of cortical regulation of emotional memory is likely to lead to new insights into how these systems might confer susceptibility or resilience to anxiety disorders. The next decade is likely to see rapid advances along several exciting new lines of research [Box 3].

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Highlights

- Prefrontal cortex is necessary for the initial learning of complex fear associations
- Trace and contextual fear paradigms offer insight into cortical control of fear learning
- Emerging evidence suggests prefrontal cortex may modulate standard fear conditioning
- We review recent findings of an expanded role for prefrontal cortex in fear regulation

Box 1. Role of the prefrontal cortex in memory storage

Activity in the prefrontal cortex is critical for the acquisition of trace and contextual fear memories, but whether the PFC also serves as a storage site for these memories is a matter of some debate. In trace fear conditioning, the initial association of the CS and UCS across a temporal gap requires prelimbic bridging activity during the acquisition session [19]. Once acquired, does the expression of fear to the CS require the PFC? Do long-term plastic changes occur at prefrontal synapses as a result of learning? Post-training or pre-testing manipulations of PFC have attempted to address these questions. Excitotoxic lesions of the PFC administered 1 day after trace fear conditioning do not prevent the successful recall of CS memory several days later [98], consistent with systems consolidation of hippocampus-dependent memory. However, consolidation of trace fear conditioning does depend on plastic changes within the PFC shortly after training. Training activates phosphorylated extracellular-regulated kinase (pERK) in the PFC, and blocking prefrontal pERK activation prior to training impairs memory formation [81]. Post-training inhibition of protein synthesis or protein degradation in PL PFC impairs the formation of trace, but not delay, fear memory [99, 100]. Moreover, memory is enhanced by post-training injection of a β 2-adrenoceptor agonist [101]. Although seemingly contradictory, these findings can be reconciled if memory storage is distributed within a network of cortical and subcortical sites. The PFC does serve as a memory storage site for trace fear conditioning, but subsequent recall of memory may be supported by hippocampus or amygdala [98, 102–104]. This raises the question of what information about trace fear conditioning is being stored in the PFC. Protein synthesis-dependent synaptic changes in PFC have been shown to be required for the consolidation of spatial working memory [105], suggesting that learning strategies or temporal relationships may be stored in prefrontal circuits. Such information is likely to contribute to behavioral flexibility in novel situations. Behavioral testing procedures that are sensitive to these more complex aspects of the memory will be needed to test these possibilities in fear memory storage.

Box 2. Working model of prefrontal regulation of memory formation

The PFC is required for associative fear learning involving temporal or contextual complexity. Recent evidence suggests that the PFC is likely recruited for learning based on its known roles in temporal bridging, attention, and assessment of cue salience or predictive value. What is not clear is how PFC integrates relevant cue and contextual information within local circuitry and how PFC then facilitates associative plasticity and memory storage in the amygdala and other regions. The hippocampus and amygdala are strong candidates for providing PFC with processed information about cues and context. Communication between the ventral hippocampus and PFC is necessary for the contextual modulation of fear extinction [15, 106] and for successful performance in spatial working memory tasks [64, 65]. Projections from the amygdala to the PFC can modulate cortical activity during discrete fear cues [22, 79, 87] and a brainstem-thalamic-prefrontal pathway may relay UCS expectancy [25].

How might the PFC integrate these diverse inputs? One possibility is the modulation of prefrontal firing by separate inputs converging onto the same prefrontal circuits. Projections from the hippocampus and amygdala converge in the PFC and the relative timing of these inputs can either enhance or attenuate the influence of each input on prefrontal firing [107]. Ventral hippocampal activation of inhibitory circuits in PFC can gate prefrontal responses to amygdala input during the presentation of an extinguished fear CS [108]. Similar modulation of prefrontal activity by converging inputs from hippocampus, amygdala, sensory cortices, or thalamus may contribute to the recruitment of PFC during more complex forms of fear learning. Another possible mechanism of prefrontal integration of diverse input is the entrainment of prefrontal firing to hippocampal theta rhythm. Synchronization of unit firing to an ongoing rhythm can organize a distributed network of distal cells [109], and this may serve to coordinate the activity of separate sets of cells, perhaps with distinct projection targets, within a larger functionally and anatomically heterogeneous population. Prefrontal units tend to fire at specific phases of the ongoing hippocampal theta rhythm during spatial working memory tasks [110, 111], with more consistent phase-locking observed on correct performance trials than on error trials [110]. Prefrontal units also exhibit phase-locking to local or hippocampal theta rhythm during appetitive or eyeblink trace conditioning [40, 112]. In fact, acquisition of trace eyeblink conditioning is enhanced when trials are delivered contingent upon hippocampal theta rhythm and sustained unit activity in PFC during the trace interval is only observed on trials delivered during hippocampal theta [40]. The PFC also exhibits increased synchronization in the local theta rhythm during a cue that predicts shock (CS+), but not a cue that predicts no shock (CS-), and this synchrony is dependent on transient disinhibition of principal neurons by parvalbumin-containing interneurons [90]. The origin of this synchronizing signal is unknown, but projections from the amygdala to the PL exhibit increased burst firing to predictive cues during fear learning [87] and may help to synchronize firing at cue onset. These findings support a model in which the PFC may integrate cue salience from the amygdala with temporal and contextual information from the hippocampus to facilitate attention or working memory to appropriate stimuli (Box Figure).

How might PFC then subsequently regulate plasticity and memory storage elsewhere? The PFC may facilitate transfer of information between other structures through neuronal synchronization, as has been observed in rhinal cortices following hippocampus-dependent learning [113]. Prefrontal-amygdala synchrony in non-human primates is associated with stronger memory and resistance to extinction in animals fear conditioned with a partial reinforcement schedule – a procedure that introduces uncertainty into the CS-UCS association [114]. Alternatively, learning-related plasticity in amygdala circuits may be achieved through phasic or tonic firing in PFC projections to the amygdala [91, 115–117]. The prefrontal cells that exhibited increased theta synchrony during the CS+ in the Courtin et al. study described above were identified as amygdala-projecting cells. Theta synchrony in these cells correlated with fear expression after learning [90]. Thus, a subset of prefrontal cells projecting to the amygdala may convey information about the predictive value of the relevant CS during learning. PFC likely regulates other inputs to the amygdala in order to modulate memory formation based on working memory or attention. Converging input from the ventral hippocampus and PFC is thought to mediate the contextual control of extinction [15], and prefrontal input to the amygdala interacts with neurotransmitter systems to modulate amygdala plasticity to fear cues [118]. A recent optogenetic study of prefrontal and hippocampal inputs to the amygdala highlights the diversity of cell-type targets, demonstrating the potential for very fine control of plasticity in amygdala circuitry by PFC and the ventral hippocampus [119].

Clearly, more work is needed to identify the mechanisms by which PFC integrates diverse emotional and associative inputs and regulates memory formation in a distributed network. The combination of fear conditioning paradigms that engage this network during learning and sophisticated optogenetic approaches are providing an avenue for testing the hypotheses of this working model.

Box 3. Future directions

- Further work is needed to determine the specific role of the PFC in contextual fear memory. Current evidence suggests that its involvement relates to appraising the predictive relationship of contextual stimuli relative to discrete stimuli [11]. The contextual or spatial component may be key as differential fear conditioning with two discrete cues does not require PFC [46]. Given the importance of the PFC in contextual modulation of fear extinction [15], the PFC may play a selective role in contextual associations. Furthermore, it remains to be determined whether ACC, PL, and IL sub-regions of the PFC differentially regulate contextual fear.
- An open question is how bridging activity in the PFC during trace fear conditioning is communicated with amygdala, hippocampus, and rhinal cortices for coordination of fear CRs and facilitation of memory formation. The PFC may provide a sustained input to BLA neurons during each trial, similar to the proposed top-down cortical input pattern hypothesized to support trace eyeblink conditioning in cerebellar circuits [38, 41]. The PFC may also coordinate persistent firing to the auditory CS in entorhinal and perirhinal cortex, both of which exhibit persistent firing properties and are necessary for trace fear conditioning [31, 43, 68, 120]. Current work in our laboratories is testing these possibilities.
- The PFC exhibits protein synthesis- and protein degradation-dependent consolidation of trace fear conditioning, similar to hippocampus and amygdala [99, 100, 102, 103], suggesting a distributed network of memory storage for these complex variants of fear conditioning. Does this distributed plasticity change the circuitry of fear extinction? Recent work from our lab suggests that this may be the case. Whereas extinction of delay fear requires NMDA receptor-mediated transmission in the amygdala, trace fear extinction is intact after intra-amygdala injection of NMDA receptor antagonists [121]. Instead, extinction of trace fear depends on both the PFC and retrosplenial cortex [121]. Determining how the extinction of trace and contextual fear memories differs from delay extinction may shed light on individual differences in clinical efficacy of extinction-based therapies for fear and anxiety disorders.

Figure 1.

Bridging activity in the PL PFC is necessary for trace fear conditioning. (A) Rats were trained with trace fear conditioning and unit activity in PFC or hippocampus was recorded during training. Prelimbic, but not hippocampal, neurons exhibit sustained bridging activity during the trace interval separating the CS and UCS. Each histogram shows the mean change in firing from baseline in 1-sec bins for a population of neurons in prelimbic PFC or dentate gyrus, averaged across several trace conditioning trials. The arrowhead indicates shock delivery. (B) Rats were injected with the inhibitory opsin ArchT into the PL PFC and

spontaneous activity was recorded in the presence or absence of laser light (532nm). The example multiunit activity trace recorded from the PL PFC of a rat previously injected with ArchT in the PL shows that brief 20s periods of light (green bar) effectively silenced spontaneous activity. (C) Rats were injected with ArchT in the PL PFC and optic fibers were implanted in the PL PFC. Rats were then trained with trace fear conditioning. The diagram shows when light (green bar) was delivered during each trial for each group. The graph shows the mean freezing during the training session. Each point shows the freezing during each trial (10-sec CS and 20-sec trace interval). (D) The following day, all rats were tested for memory of the CS in a novel chamber in the absence of laser light. The graph shows the freezing response during the CS. Light delivered during the trace interval or whole trial the previous day impaired learning relative to control rats, showing that trace interval activity in PL links the CS and UCS in memory. * $p < 0.05$. Adapted with permission from [36, 37] (A) and [19] (B–D).

Figure 2.

Distinct activation patterns in trace and delay conditioning in frontal cortical regions. (A) Subjects were trained in a differential fear conditioning procedure in which a delay conditional CS was paired with shock (CS+), a trace conditioning CS was paired with a shock delivered after a 10-sec trace interval (CS₁₀), and a third CS was never paired with shock (CS-). Activation related to forming the CS-UCS association (i.e., trace and delay, but not CS-) was observed in anterior cingulate. The histograms depict the baseline-adjusted area under the impulse response curve (AUC) measured for each stimulus within each region of interest. The response magnitude within these regions was significantly larger

during the CS+ and trace interval than during CS– presentations. (B) Activation specific to the trace interval was observed in the middle frontal gyrus (a dorsolateral PFC area). The response magnitude within this region was larger during the trace interval than during the CS+, CS₁₀, and CS–. Additional areas activated during the trace interval may reflect other cortical processes specific to a delay period, such as motor preparation in anticipation of UCS delivery (the supplementary motor area; SMA) or timing of the sensory UCS (frontal operculum) [discussed in 27]. Reproduced with permission from [27] (C) Summary figure highlighting the functional uniqueness of various cortical areas in fear conditioning, observed across several studies of trace fear conditioning [27, 53, 69].

Box2Fig.