Review

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Prefrontal cortical regulation of fear learning

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The prefrontal cortex regulates the expression of fear based on previously learned information. Recently, this brain area has emerged as being crucial 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 the prefrontal cortex is recruited in the formation of memory, highlighting relevant work in laboratory animals and human subjects. We propose that the prefrontal cortex facilitates fear memory through the integration of sensory and emotional signals and through the coordination of memory storage in an amygdala-based network.

An expanded role for the prefrontal cortex in fear memory

Adaptive responding to threat is crucial 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 threats or inappropriate fear in non-threatening situations. Because approximately 18% of the US 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 the accurate prediction of an aversive outcome from available environmental signals. For this reason, fear conditioning has proved to be a powerful tool for investigating the neurobiology supporting emotional learning and fear expression in both human and non-human subjects. Fear conditioning studies have described critical roles for the amygdala, hippocampus, and cerebral cortex in 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 in the subject, including changes in autonomic activity, analgesia, and freezing behavior. The power of this procedure comes from its simplicity, rapid acquisition, translational application, and sensitivity to cellular/molecular-, genetic-, and systems-level manipulations. 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]. Over 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]. The ventral PFC in the rat seems to be necessary for controlling fear to a CS that no longer predicts danger, as in extinction learning [7,8]. By contrast, the 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 crucial 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 seems to be 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 versus fear learning

The PFC was initially implicated in emotional regulation on the basis of 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, such as a light or 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

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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 the 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: that is, coordinating action through the 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 the 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 over 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, the 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 might 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 PFC, 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. Although some computational models suggest that the hippocampus might provide a bridging signal [34,35], neither the CA1 nor dentate gyrus (DG) areas exhibit firing patterns that are 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 the PFC maintain firing past CS offset and into the trace interval for both short (2 s [24]) and long (20 s [37]) intervals (Figure 1A). These 'bridging' cells are observed in the dorsal, prelimbic area (PL), but not the ventral, infralimbic area (IL) [37]. Similar results have been obtained in rabbits performing trace eyeblink conditioning, with persistent firing neurons located primarily in deep, output layers of the dorsal PL and anterior cingulate cortex (ACC) [38–40]. This anatomical position is in line with a model in which the PL provides a bridging signal, allowing CS-activated networks to coincide with UCS delivery. Elegant work by the Mauk laboratory 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 conditional fear responses 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 that are associated with the persistent firing of cortical cells, such as the 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 PL neurons during the trace interval [19]. Silencing PL activity during the 20 s 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 might reflect the maintenance of attentional resources during the CS–UCS interval and/or the coordination of associative encoding downstream in the 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 the PFC in contextual learning is less clear. Contextual fear conditioning is largely supported by the hippocampus and amygdala. Lesions or inactivation of the 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,