Prefrontal Cortex in Learning to Overcome Generalized Fear

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SUMMARY: Normal brain functioning relies critically on the ability to control appropriate behavioral responses to fearful stimuli. Overgeneralized fear is the major symptom of anxiety disorders including posttraumatic stress disorder. This review describes recent data demonstrating that the medial prefrontal cortex (mPFC) plays a critical role in the refining of cues that drive the acquisition of fear response. Recent studies on molecular mechanisms that underlie the role of mPFC in fear discrimination learning are discussed. These studies suggest that prefrontal N-methyl-D-aspartate receptors expressed in excitatory neurons govern fear discrimination learning via a mechanism involving cAMP response element-binding protein–dependent engagement of acetyltransferase.

KEYWORDS: fear discrimination, prefrontal cortex, overgeneralization, NMDA Receptor, CBP

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The concepts of fear memory accuracy and generalization reflect the ability of a subject to respond properly to a similar stimulus to that of the trained stimulus presented during fear conditioning procedure. Specifically, the ability to distinguish between these two stimuli indicates the level of fear memory accuracy (discrimination), whereas elevated level of fearful responses to harmless stimuli is an indicative of fear generalization. Fear memory accuracy is critical for survival, while fear generalization is effective for recalling, threat assessment, and avoiding dangerous situations. An extreme or excessive fear of stimuli that are not harmful is referred to as fear overgeneralization. Overgeneralized fear is the major symptom of anxiety disorders including phobia, panic disorders, generalized anxiety disorder,[1,2] and posttraumatic stress disorder,[3] triggered by secure environment cues resembling those of the traumatic experience. Fear behavior is controlled by adaptive processes including discrimination, generalization, and extinction. These concepts were initially developed by Pavlov[4] and have been extensively studied for a century. Discriminatory fear learning involves fear conditioning, which is a form of classical Pavlovian conditioning[4] and has become the best studied behavioral model for associative learning and its underlying synaptic and circuit-level plasticity.[5–7] Multiple memory systems theory postulates that different types of memory are consolidated via hardwired pathways.[8] In tone fear conditioning, tone [conditional stimulus (CS)]-foot shock [unconditional stimulus (US)] associations are directly encoded through synaptic plasticity in the amygdala, which receives direct auditory inputs. During the contextual fear conditioning, the contextual stimulus (CS) is encoded by the dorsal hippocampus (and later consolidated by the hippocampus–prefrontal circuitry), whose outputs are subsequently associated with the US through synaptic plasticity in the amygdala.[5–7]

The fact that expression of recent and remote long-term fear memories requires the dorsal hippocampus and medial prefrontal cortex (mPFC), respectively,[9,10] suggests that the communication between these two brain regions controls transition from a recent state to remote state during system-level memory consolidation. Both regions appear to be engaged in context coding. In fact, context-specific neuronal ensembles were found in both regions.[11,12] While the dorsal hippocampus and mPFC appear to track spatial information, the mPFC is likely to integrate contextual recognition of fear–context association with distinctive roles of infralimbic (IL) and prelimbic (PL) subregions of the mPFC.[13,14] Fear behavior is differentially regulated by PL and IL of the mPFC[15–18] via increase or decrease of fear expression, respectively,[18,19] which may be due to differential connectivity with the amygdala (Fig. 1).[20–22] This differential role of IL versus PL is of particular interest since, under the Hull-Spence theory of discrimination learning postulates, conditioned excitation (the result of reinforcement) and inhibition (the result of nonreinforcement) are postulated to have generalization gradients.[23–25] The algebraic summation of excitatory and inhibitory strength determines the response rate to test stimuli.[26] For example, differential

conditioning increases unit and field responses to the conditioned stimulus (CS+), reinforced with an electric shock CS+, whereas responses to the second stimulus that was nonreinforced (CS–) decreased.[27] Both excitatory and inhibitory neurons in the mPFC play important roles in the regulation of fear responses. For example, direct inhibition of fast-spiking interneurons in the dorsomedial prefrontal cortex disinhibits prefrontal excitatory neurons and promotes fear expression.[28] The mPFC can compensate for absence of dorsal hippocampus in contextual fear learning, while IL mPFC lesions enhance generalization of contextual fear and interfere with discriminatory fear learning.[29] In addition, the interaction between the thalamus and mPFC has been implicated in the contextual fear generalization[30,31] and the retrieval of long-term fear memories.[32]

That the mPFC is a locus for gating fear discrimination and danger assessment is supported by additional evidence that includes animal studies in which an mPFC lesion impairs the ability to guide behavior, specifically when memory retrieval resolves conflicting dangerous and harmless contextual cues.[33–36] A fear decline is associated with elevated activity in the mPFC as determined by activation of immediate-early genes,[37,38] blood oxygenation levels,[39] cell firing,[40] and local field potentials.[41] The mPFC has dense reciprocal anatomical and functional connections with sensory cortices, thalamic sensory relays, and memory systems including the hippocampus (context, multisensory processing) and the amygdala, the critical locus for fear processing. Considerable evidence indicates that neurons in mPFC, basolateral amygdala (BLA), and hip are functionally coupled at the theta range (4–12 Hz oscillations) during fear conditioning,[42,43] conditioned extinction,[41] and discriminative fear learning.[44] Moreover, memory retrieval elicits mPFC neuronal activity patterns reminiscent of neural representations of behavioral contexts that govern successful recollection. Different behavioral contexts evoke distinct firing of neuronal ensembles,[12] which can reset during uncertainty following environmental change[45] or induce sudden transitions between neural ensemble states accompanied by behavioral transitions.[46]

Multiple memory systems theory postulates that different types of memory are consolidated via hardwired pathways;[8] however, how memories are integrated[47,48] to specific neurons and synapses in a circuit remains unclear. While the circuit-, cellular-, and molecular-level mechanisms of fear extinction and contextual conditioning have been studied in the mPFC, much less is known about how the neural circuitry of the mPFC contributes to fear discriminatory learning. Glutamate, the main excitatory neurotransmitter is critically involved in fear memory.[49] In addition, it has been demonstrated that specific glutamate postsynaptic receptors such as N-methyl-D-aspartate receptors (NMDARs) are directly involved in various learning mechanisms including modulation of fear memory.[50–53] Furthermore, NMDAR, Ca^{2+} signaling, transcription factor cAMP response element-binding protein (CREB), and CREB-binding protein's (CBP's) intrinsic histone acetyltransferase activity (HAT) have been implicated as putative mechanisms for long-term memory encoding into cortical circuits.[54–56] Using fear discrimination learning assays, it was recently demonstrated that NMDAR-, CREB-, and CBP's HAT-dependent signaling in the mPFC is required for successful fear discrimination learning (Fig. 2).[57,58] Thus, fear discrimination is attained via the mPFC-dependent reduction of generalized fear responses to harmless stimuli.[57,58] Both selective inhibition of CBP HAT or CREB function in the mPFC circuitry show strong deficit in fear discrimination learning.[58] A similar effect was observed in mice with selective deletion of NMDAR from excitatory neurons in the mPFC.[57] These data suggest that successful fear discrimination involves prefrontal NMDAR-dependent mechanism governing decline of generalized fear responses to harmless nonreinforced stimuli.

While both CBP and CREB are partners and both are implicated in long-term plasticity and memory consolidation in Aplysia, Drosophila, and mice, CREB has been strongly implicated in adaptive alteration of neuronal excitability and memory allocation[48] and it is possible that CBP HAT may mediate CREB-dependent changes in neuronal excitability. Four independent manipulations to downregulate CBP acetyltransferase activity specifically in an adult living brain to avoid development confound have been reported.[55,59,60] Histones are believed to be the primary targets for CBP's HAT activity; however, a number of nonhistone targets for CBP's HAT activity, which are involved in chromatin remodeling and gene expression regulation, have been discovered.[61–67] While the impact of histone

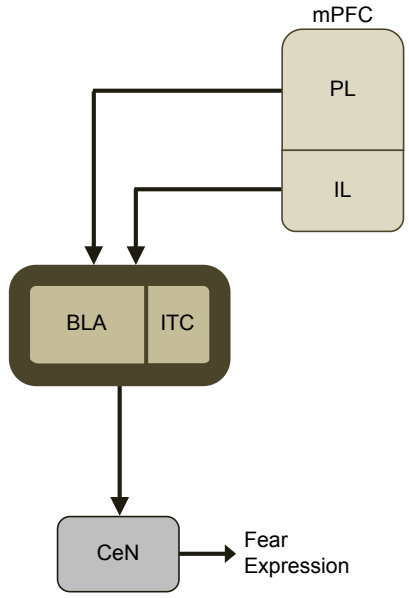

Figure 1. Fear behavior is differentially regulated by IL and PL subregions of the mPFC (see text).
Notes: Complex interactions and circuitry for fear discrimination learning also comprise the basolateral nucleus of the amygdala (BLA), the central nucleus of the amygdala (CeN), and the amygdala intercalated neurons (ITC).

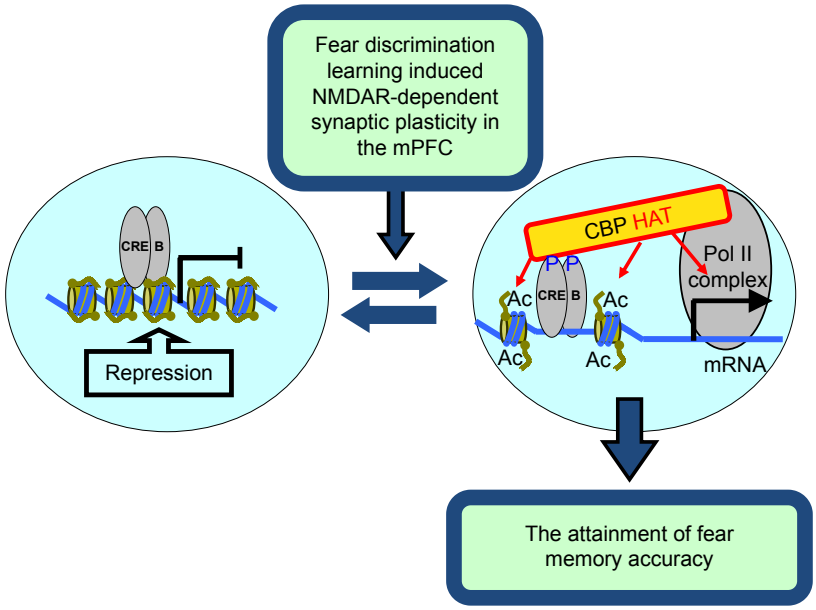

Figure 2. Model of acetylation-dependent fear discrimination learning.
Notes: In the absence of appropriate stimulation, the target genes are repressed. Recent studies reveal that NMDARs expressed in prefrontal excitatory neurons control the ability to distinguish between dangerous and harmless stimuli via a mechanism that involves CREB-dependent engagement of acetyltransferase. This model suggests that NMDAR-induced plasticity, NMDAR-dependent CREB phosphorylation at Serine-131, and NMDAR-dependent CREB-dependent engagement of CBP acetyltransferase control the consolidation of specific memories required for successful fear discrimination learning.
Abbreviations: Ac, acetylated histone; Pol II complex, Polymerase II complex.

and nonhistone protein acetylation by CBP is not fully understood, CBP's HAT appears to be a critical component of a putative epigenetic mechanism that controls long-term memory.[55,60]

Recent data indicate that the mPFC plays a critical role in the refining of cues that drive the acquisition of fear response, including some molecular mechanisms that underlie this role. These data are in line with previous work showing that the mPFC supports fear extinction, because fear discrimination involves selective reduction of the response to nonreinforced stimuli, perhaps through interaction between the amygdala, hippocampal system, and mPFC during a consolidation of selective memories. The findings indicating that three components of the molecular mechanism underlying long-term plasticity in the mPFC (NMDAR, CREB and CBP HAT) are directly implicated in appropriate disambiguation of fear signals provide direct evidence that fear discrimination involves long-term memory coding into the prefrontal excitatory circuitry. Modern neuroscience now has the tools to begin to characterize the mechanisms and neural circuits responsible for fear memories remaining distinct and resistant to confusion, and further experiments are needed to reveal what type of information is consolidated in the mPFC that is required for the attainment of fear memory accuracy after initial fear generalization.

Author Contributions

Conceived and designed the experiments: EK. Analyzed the data: EK. Wrote the first draft of the manuscript: EK. Contributed to the writing of the manuscript: EK. Agree with manuscript results and conclusions: EK. Jointly developed the structure and arguments for the paper: EK. Made critical revisions and approved final version: EK. Author reviewed and approved of the final manuscript.

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