Prefrontal Mechanisms in Extinction of Conditioned Fear

Gregory J. Quirk, René Garcia, and Francisco González-Lima

Interest in the medial prefrontal cortex (mPFC) as a source of behavioral inhibition has increased with the mounting evidence for a functional role of the mPFC in extinction of conditioned fear. In fear extinction, a tone-conditioned stimulus (CS) previously paired with a footshock is presented repeatedly in the absence of footshock, causing fear responses to diminish. Here, we review converging evidence from different laboratories implicating the mPFC in memory circuits for fear extinction: (1) lesions of mPFC impair recall of extinction under various conditions, (2) extinction potentiates mPFC physiological responses to the CS, (3) mPFC potentiation is correlated with extinction behavior, and (4) stimulation of mPFC strengthens extinction memory. These findings support Pavlov's original notion that extinction is new learning, rather than erasure of conditioning. In people suffering from posttraumatic stress disorder (PTSD), homologous areas of ventral mPFC show morphological and functional abnormalities, suggesting that extinction circuits are compromised in PTSD. Strategies for augmenting prefrontal function for clinical benefit are discussed.

Key Words: Amygdala, infralimbic, long-term potentiation, prelimbic, PTSD

The study of fear and anxiety in experimental animals has advanced rapidly with the use of Pavlovian fear conditioning, in which a tone-conditioned stimulus (CS) is associated with a footshock unconditioned stimulus (US). Conditioned fear reactions to the tone extinguish in the absence of the shock. The resurgence of interest in extinction is due in large part to its potential applicability to the treatment of anxiety disorders, such as posttraumatic stress disorder (PTSD), in which extinction is thought to be compromised. A thorough understanding of the neural circuits of extinction of fear could yield new treatments for augmenting exposure-based therapies that are used to treat PTSD (Anderson et al 2004; Ressler et al 2004).

In his classic investigation of appetitive conditioning in dogs, Pavlov observed that extinguished responses spontaneously recovered with the passage of time (Pavlov 1927). This suggested that extinction did not erase the memory for conditioning but represented new learning. More recent behavioral studies have confirmed and extended this finding for conditioned fear (Bouton 2002; Quirk 2002; Rescorla 2004; Rescorla and Heth 1975). If extinction does not erase the conditioning memory, it must form a new memory that inhibits the conditioned response. This suggests that some structure or structures are activated by extinction, so as to excite inhibitory circuits that are responsible for reducing the expression of fear (Figure 1). Despite early theoretical formulations of extinction-related inhibition (Konorski 1967; Pavlov 1927), the search for inhibitory circuits largely has been unsuccessful (Chan et al 2001; Kimble and Kimble 1970). However, studies that build on recent advances in the acquisition of conditioned fear point to the medial prefrontal cortex (mPFC) as an important part of the neural circuit for fear extinction. In this review, we describe converging evidence from lesion, recording, metabolic, stimulation, and microinfusion studies in rodents supporting this hypothesis. We also suggest ways in which prefrontal mechanisms of extinction may be augmented so as to enhance extinction, with potential clinical applications.

From the Department of Physiology (GJQ), Ponce School of Medicine, Ponce, Puerto Rico; Neurobiologie et Psychopathologie (RG), Universite de Nice-Sophia Antipolis, Nice, France; and the Institute for Neuroscience and Department of Psychology (FGL), University of Texas at Austin, Austin, Texas.

Address reprint requests to Gregory J. Quirk, Ph.D., Department of Physiology, Ponce School of Medicine, P.O. Box 7004, Ponce, Puerto Rico 00732; E-mail: gjquirk@yahoo.com.

Received August 1, 2005; revised October 16, 2005; accepted March 3, 2006.

Lesion Studies

The idea that extinction circuits involved the prefrontal cortex originated with early primate studies of appetitive conditioning, in which lesions of the ventral mPFC (vmPFC) and orbitofrontal cortex resulted in increased responding during extinction (Butter et al 1963; for a complete history of prefrontal cortex in extinction, see Sotres-Bayon et al 2006, in this issue). Later, Morgan and colleagues (Morgan et al 1993; Morgan and LeDoux 1995) observed that rats with vmPFC lesions could acquire fear normally but had difficulty extinguishing across several days of extinction training. Quirk and colleagues (2000) then showed that rats with vmPFC lesions that were centered on the infralimbic cortex (IL) could extinguish normally within a session but had difficulty recalling extinction 24 hours later, suggesting that IL is not required for fear inhibition under all circumstances but is important for recalling extinction after a long delay. Other studies have confirmed that vmPFC lesions impair recall of extinction in aversive (Lebron et al 2004; Morgan et al 2003; Morrow et al 1999; Weible et al 2000) and appetitive (Rhodes and Killcross 2004) conditioning.

Recording Studies

Lesion studies presuppose that regional contributions to brain function may be inferred from a damaged brain. A more direct approach is to record from neuronal activity in awake animals undergoing extinction training. Do mPFC neurons signal extinction? Paralleling mPFC lesion findings, single neurons in IL did not signal the tone CS during acquisition or extinction training (Milad and Quirk 2002). The next day, however, when rats were recalling extinction, IL units showed potentiation of short-latency tone responses (Figure 2). The larger the tone response, the lower the spontaneous recovery of freezing, consistent with IL-mediated inhibition of fear after extinction. No such potentiation was observed in adjacent prelimbic cortex. Thus, extinction potentiated auditory inputs to IL neurons, providing direct support for the Pavlov-Konorski hypothesis that extinction potentiates neuronal activity in structures that are involved in inhibition of the conditioned response (Konorski 1967; Pavlov 1927).

What inputs to mPFC might become potentiated as a result of extinction? To address this, Garcia and colleagues determined whether repeated presentations of a tone CS in the absence of

0006-3223/06/$32.00
doi:10.1016/j.biopsych.2006.03.010

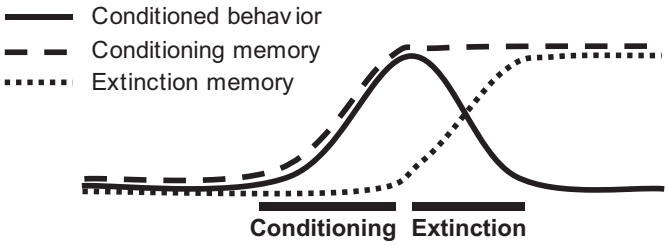

Figure 1. Schematic relating conditioned behavior to memory for conditioning and extinction. As first suggested by Pavlov, extinction training does not eliminate memory for conditioning but generates a new memory that competes with conditioning for control of behavior. For conditioned fear, this schema suggests that there are structures in the brain that increase their neuronal activity with extinction, so as to drive down fear via inhibition of fear expression centers.

the US induces long-term potentiation (LTP) in the mPFC. The mPFC receives glutamatergic inputs from the hippocampus (Jay and Witter 1991), the mediodorsal thalamus (MD; Pirot et al 1994), and the basolateral amygdala (BLA; McDonald 1991). High-frequency stimulation of each of these input areas results in LTP in mPFC-evoked potentials (Herry et al 1999; Jay et al 1995; Maroun and Richter-Levin 2003). So far, analyses of two of these pathways have confirmed development of LTP-like changes in the mPFC with extinction training. MD-evoked responses in mPFC show little change during extinction training but are increased 1–7 days after extinction (Herry et al 1999; Herry and Garcia 2002). Similarly, extinction-related LTP takes place in the hippocampal–mPFC pathway after extinction training (Farinelli et al, in press). Interestingly, failure to recall extinction was associated with inhibition of MD-evoked potentials, and depressing the MD-mPFC pathway with low-frequency stimulation caused full recovery of conditioned fear after extinction (Herry and Garcia 2002, 2003). Thus, extinction training results in LTP of thalamic inputs to mPFC even days after extinction, paralleling the single-unit recording studies (Milad and Quirk 2002) and indicating a role of mPFC in long-term retention of extinction memory. Thus, inputs to the mPFC from the thalamus, hippocampus, or the BLA may become potentiated after extinction.

Microinfusion data have strongly implicated the BLA in acquisition of extinction (Lin et al 2003; Myers and Davis 2002); however, lesions of the basal nucleus have no effect on short- or long-term memory for extinction (Anglada-Figueroa and Quirk 2005; Sotres-Bayon et al 2004). This highlights the potential importance of the lateral amygdala in the acquisition of extinction. Another potentially important input to mPFC is the auditory association cortex (Condé et al 1995), in light of reports that auditory-cortex lesions impair extinction of auditory fear conditioning (Song and Kim 2004; Teich et al 1989). Local inactivation of various inputs to mPFC is needed to determine which ones may impair extinction learning and memory.

Metabolic Mapping

In addition to single-unit and evoked potential recording, extinction of auditory conditioning also has been investigated with metabolic-mapping techniques that assess the uptake of fluorodeoxyglucose (FDG), a radiolabeled glucose analog (Barrett et al 2003). Brain activity can be mapped with FDG because brain cells use glucose and its analogs for energy metabolism (Sokoloff 1992). An important advantage of metabolic mapping over electrophysiological recording methods is that the entire brain can be examined at once, permitting visualization of behavioral networks. Metabolic responses to a test tone were compared in groups of mice that received fear conditioning, a pseudorandom treatment (unpaired tones and shocks), or conditioning followed by extinction. Consistent with single-unit and evoked-potential recording, the largest increase in metabolic activity after extinction occurred in the mPFC. The infralimbic (but not the prelimbic) area showed significantly more metabolic activity than controls. In addition to IL, significant metabolic increases were observed in dorsal, medial, and lateral frontal cortex, which are areas not yet studied with the unit-recording technique. Hence, multiple prefrontal regions may play a role in extinction memory. There also were changes in the interaction between the prefrontal cortex and other regions, particularly in auditory and limbic networks. In support of an inhibitory role, FDG labeling in dmPFC, in IL cortex, and in dorsal and lateral frontal cortex was correlated significantly with extinction behavior (Barrett et al 2003). Finally, there was a strong negative correlation between prefrontal areas and regions thought to be involved in expression of conditioned fear, such as the ventral tegmental area, MD thalamus, and the entire auditory system (brainstem, thalamic, and cortical levels; Barrett et al 2003).

These mapping data suggest that extinction training engages a network of interactive brain regions, which may serve two functions: to inhibit the conditioned response after extinction and to preserve some of the original CS-US associative effects

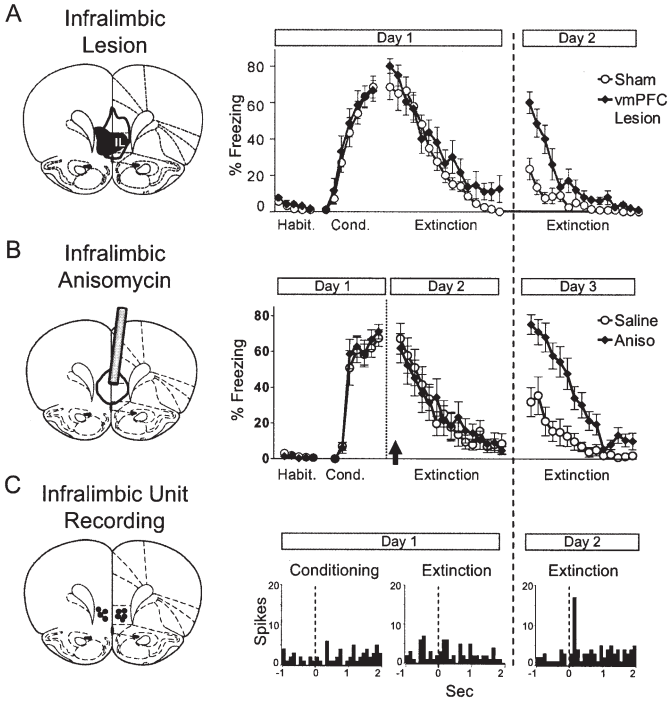

Figure 2. Converging lines of evidence showing that the infralimbic prefrontal cortex (IL) is functionally involved in recall of extinction. (**A**) Lesions of IL do not prevent extinction but interfere with recall of extinction the following day (modified from Quirk et al 2000). (**B**) Unit recording shows that IL neurons respond to the tone only during recall of extinction, suggesting that IL tone responses are responsible for low fear after extinction (modified from Milad and Quirk, 2002). (**C**) Infusing the protein synthesis inhibitor anisomycin (Aniso) into the IL just before extinction (arrow) has no effect on extinction learning but blocked recall of extinction the following day (modified from Santini et al 2004). These and other data suggest that extinction-induced potentiation of prefrontal neuronal activity is necessary for suppression of fear after extinction. vmPFC, ventral medial prefrontal cortex; Habit., habituation; Cond., conditioning.

Table 1. Converging Lines of Evidence from Recent Rodent Studies Showing that Extinction can be Facilitated by Activation of Medial Prefrontal Cortex (mPFC)

MPFC Activity is Enhanced by Extinction Training	MPFC Activity Is Correlated with Extinction Behavior	Increasing mPFC Activity Strengthens Extinction
Single-unit responses to CS[a]	Single-unit responses to CS[a]	Electrical stimulation paired with CS[a,e]
Field potentials evoked by thalamic stimulation[b]	Field potentials evoked by thalamic stimulation[d]	Long-term potentiation of thalamic inputs[d]
Metabolic activity to CS[c]	Metabolic activity to CS[c]	Metabolic enhancement with methylene blue[f]

CS, conditioned stimulus.
[a]Milad and Quirk (2002).
[b]Herry and Garcia (1999).
[c]Barrett et al (2003).
[d]Herry and Garcia (2002).
[e]Milad et al (2004).
[f]Gonzalez-Lima and Bruchey (2004).

from acquisition. Thus, there is remarkable convergence between the three different techniques (single-unit, evoked potential, metabolic mapping) in two species (rat and mouse), showing that extinction potentiates vmPFC responses to the tone CS (Table 1). These results clearly support Pavlov's cortical inhibition hypothesis and contradict the simpler notions of extinction as unlearning or reversal of acquisition.

Molecular Studies

Formation of long-term memory has been linked to a molecular cascade involving N-methyl-D-aspartate (NMDA)-mediated calcium entry, activation of protein kinases, gene expression, and protein synthesis (Kandel 2001). Involvement of this cascade in extinction would provide support for the idea that extinction constitutes new learning. It has been known for some time that blocking NMDA receptors systemically (Baker and Azorlosa 1996; Cox and Westbrook 1994; Santini et al 2001) or within the amygdala (Falls et al 1992; Walker and Davis 2002) prevents the formation of long-term memory for extinction. Protein kinases and protein synthesis in the amygdala also have been implicated in extinction (Lin et al 2003; Lu et al 2001).

Recent evidence suggests that a similar molecular cascade operates in the mPFC during extinction. Antagonists of NMDA receptors (Burgos-Robles et al 2004), mitogen-activated protein kinases (MAPk; Hugues et al 2004), or protein synthesis (Santini et al 2004) prevent the formation of long-term (but not short-term) extinction when microinfused into the mPFC. In each case, delaying the infusion 2 or 4 hours after extinction eliminated the effect, consistent with a time-limited role of molecular processes in consolidation of extinction. Western blot analysis of prefrontal tissue shows that infusion of MAPk inhibitor PD098059 into the mPFC immediately after extinction decreased levels of phosphorylated ERK2 without affecting total ERKs (Hugues et al, in press). Future experiments will determine whether inhibition of extracellular signal-regulated kinase-2 (ERK2) phosphorylation is related to LTP in the MD-mPFC or hippocampal–mPFC pathways or to other inputs to the mPFC (for example, from the BLA; see Maroun and Richter-Levin, 2003).

Does activation of the ERK-MAPk system in the mPFC trigger gene expression necessary for extinction memory? Although little is known about extinction-induced gene expression, it recently was shown that extinction training stimulates the immediate early gene c-Fos in the mPFC (Mickley et al 2005; Santini et al 2004). Controls indicated that this up-regulation was not a result of tone stimulation or acquisition of fear conditioning. c-Fos is a marker of cellular activity but also can act as a transcription factor when dimerized with c-Jun (Kaczmarek 2002). These findings are consistent with a role of gene expression in extinction memory, although transcription inhibitors and transgenic approaches will be needed to determine whether gene expression is necessary for extinction memory.

Expression of Extinction

Once potentiated, how does mPFC inhibit fear after extinction? The infralimbic subregion of mPFC has extensive projections to the amygdala, as well as the amygdala's targets in the hypothalamus and brainstem (Floyd et al 2001; Hurley et al 1991; Vertes 2004). If these projections are inhibitory, the IL could override amygdala-generated fear responses. The physiological effect of many of these projections is not known, but anatomical support exists for IL-mediated inhibition of the amygdala. IL projects robustly to the region between the central and basolateral nuclei, containing intercalated (ITC) cells (Cassell and Wright 1986; McDonald et al 1996). ITC cells are gamma-amino-n-butyric acid (GABA) ergic neurons (Paré and Smith 1993a) that project to the central nucleus (Paré and Smith 1993b) and are responsible for feed-forward inhibition of central nucleus output neurons (Royer et al 1999). In support of this model, electrical stimulation of the IL area decreased the excitability of brainstem-projecting neurons of the amygdala central nucleus (Quirk et al 2003) and decreased the expression of conditioned fear (Milad et al 2004). According to this model (Figure 3), extinction-induced potentiation of tone responses in IL neurons would cause feed-forward inhibition of the central nucleus, thereby preventing fear signals in BLA from exiting the amygdala. Consistent with this, it recently was shown that chemical stimulation of IL

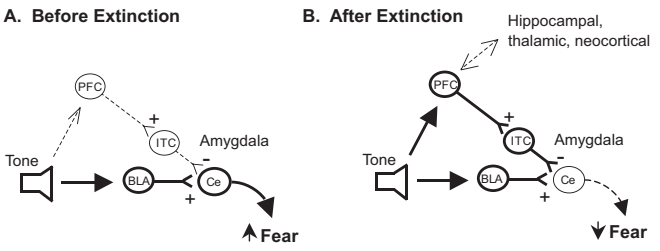

Figure 3. Schema for mPFC inhibition of fear via the amygdala. (**A**) Before extinction, the tone CS activates the basolateral amygdala (BLA), which activates the central nucleus (Ce) output neurons, triggering fear responses. (**B**) After extinction, prefrontal (PFC) responses to the tone are potentiated, which activates GABAergic intercalated cells (ITC) within the amygdala. ITC inhibition of the Ce competes with BLA excitation of Ce, effectively canceling fear responses. Potentiation of PFC responses to the CS and inhibition of conditioned fear responses also may involve reciprocal PFC interactions with hippocampal, thalamic, and neocortical pathways. Modified with permission from Milad et al (2004).

increased c-Fos expression in amygdala ITC cells (Berretta et al 2005). ITC cells also exhibit NMDA-mediated plasticity (Royer and Paré 2002), suggesting that they may participate in long-term storage of extinction.

Conflicting Lesion Evidence on the Role of the mPFC in Extinction

Although there is much physiological evidence in favor of a functional role of mPFC in learning and expression of extinction, there also are conflicting lesion reports. Two groups did not find any effect of pretraining mPFC lesions on extinction of conditioned fear (Gewirtz et al 1997; Vouimba et al 2000), whereas another study found that lesions made after conditioning did not impair subsequent extinction (Morgan et al 2003). Interpretation of permanent lesion effects often is hampered by potential recovery of function or compensation by other structures. There is a pressing need, therefore, for studies that use temporary inactivation of mPFC via microinfusion of local anesthetics or the GABA antagonist muscimol. Preliminary reports using these techniques are conflicting, showing increased fear (Corcoran and Maren 2003), decreased fear (Sierra-Mercado et al 2005), or no effect (Myers and Davis 2004) in rats recalling extinction. A challenge for future studies will be to identify the factors that could account for variability between laboratories. These might include contextual variables (e.g., AAA vs. ABB designs), the presence of a competing appetitive instrumental response (such as bar-pressing for food), or the number of extinction trials (e.g., overtraining-induced masking of effects). Another possible reason for negative lesion effects is that the mPFC likely is part of a network of structures that collectively consolidate and express extinction memory (Barrett et al 2003). Disconnection of a sufficient number of structures within the network may be a prerequisite for observing lesion deficits. Finally, recent studies show that mPFC neurons can signal acquisition of fear conditioning (Baeg et al 2001; Laviolette et al 2005) and excite neurons in the BLA (Likhtik et al 2005), suggesting that there may be separate modules within mPFC for exciting versus inhibiting fear.

Enhancing Prefrontal Function Strengthens Extinction

If prefrontal activation is essential for extinction learning, then stimulating prefrontal cortex should strengthen extinction. Support for this idea comes from experiments using electrical stimulation and metabolic enhancers. Electrical stimulation was used to mimic short-latency tone-evoked responses of infralimbic neurons (100–400 ms after tone onset; Milad et al 2004; Milad and Quirk 2002). Pairing this brief IL stimulation with conditioned tones reduced the expression of freezing, consistent with feed-forward inhibition of amygdala output neurons (Quirk et al 2003). mPFC stimulation also strengthened extinction learning as evidenced by persistent decreased fear responses the day after the stimulation, suggesting LTP of extinction-related synapses in mPFC.

The role of LTP was tested directly by enhancing mPFC responsiveness to MD thalamic inputs by applying high-frequency stimulation before extinction training (Herry and Garcia 2002). MD stimulation had no effect on the rate of extinction learning within the training session, supporting lesion and unit-recording findings that mPFC is not responsible for short-term extinction memory. One week later, however, retention of extinction was markedly improved in potentiated rats, as evidenced by low rates of spontaneous recovery of freezing. Improvement in extinction retention was correlated with potentiation of mPFC evoked potentials. Thus, mPFC LTP prevented the spontaneous recovery of conditioned freezing that normally is observed with the passage of time.

An additional approach to enhancing mPFC function is the use of metabolic enhancers such as methylene blue (MB), which improve activity-dependent brain energy production by targeting mitochondrial oxidative metabolism (Callaway et al 2002). A memory-improving action of MB in rats first was demonstrated for inhibitory avoidance learning (Martinez et al 1978). Gonzalez-Lima and Bruchey (2004) investigated whether postextinction administration of MB could enhance retention of an extinguished conditioned response. Postextinction freezing was 50% lower in rats that were receiving 4 mg/kg of MB, a dose that chronically is used in human beings without negative side effects (Naylor et al 1986). Control rats injected with MB showed no changes in motor activity or general fearfulness, suggesting that postextinction MB administration specifically enhanced memory for extinction. Rats with improved retention of extinction also showed a greater relative increase in cytochrome oxidase activity in the same prefrontal cortical regions that are activated during extinction recall (Barrett et al 2003). Thus, MB improved extinction by augmenting extinction-induced potentiation of mPFC. Note the parallel with electrical stimulation and unit-recording findings (Table 1). Conversely, decreases in cytochrome oxidase activity in the prefrontal cortex produced by genetic selection of rats that are predisposed to helplessness (Shumake et al 2000) results in rats with deficits in fear extinction that simulate the PTSD behavioral phenotype (Shumake et al 2005).

Relevance to Treatment of Psychiatric Disorders

There is great interest in finding more effective treatments for anxiety disorders, which are among the most common mental health problems. Extinction deficits have been implicated as a possible risk factor for the development of PTSD (Charney 2004; Lissek et al 2005; Milad et al 2005). People suffering from PTSD show reduced extinction of aversively conditioned responses (Charney et al 1993; Peri et al 2000) and show impairments in a functional network involving the amygdala and anterior cingulate (Gilboa et al 2004; Shin et al 2001). Brain-imaging studies of PTSD patients show reduced activity (Bremner 2002; Shin et al 2004) and reduced volume (Rauch et al 2003) in the perigenual prefrontal cortex, an area that is homologous with extinction-related regions of rodent mPFC (Milad et al 2006). These studies also show increased amygdala activity in PTSD patients who are exposed to traumatic stimuli (Bremner 2003; Shin et al 2004), suggesting a lack of top-down control of the amygdala by structures involved in extinction of fear.

Several recent functional imaging and volumetric studies demonstrate that extinction activates perigenual and associated regions of prefrontal cortex in human beings (Gottfried and Dolan 2004; Milad et al 2005; Phelps et al 2004). In particular, Rauch and coworkers demonstrated that retention of fear extinction was correlated with the thickness of the vmPFC (Milad et al 2005), suggesting that the likelihood of developing PTSD depends on the integrity of the prefrontal extinction system. For a complete review of the human literature on extinction, see Rauch et al (2006, in this issue).

Behavioral therapy for PTSD (exposure therapy) is based mainly on the process of extinction (Hermans et al 2005). Therefore, methods of facilitating extinction and preventing the return of fear may lead to more effective therapeutic interventions. Current behavioral techniques such as flooding and implosion could be improved by pharmacological interventions that accelerate and strengthen extinction. For example, a reduction in

the number of exposure sessions required to successfully extinguish fear responses could counteract the relatively high dropout rate that is observed with this type of therapy (van Minnen and Hagenaars 2002).

Several recent studies have shown that extinction learning in rats can be accelerated and strengthened with systemically applied drugs. These include the noradrenergic antagonist yohimbine (Cain et al 2004), the dopamine D2 receptor antagonist raclopride (Ponnusamy et al 2005), the cannabinoid reuptake inhibitor AM404 (Chhatwal et al 2005), and the NMDA receptor partial agonist D-cycloserine (DCS; Ledgerwood et al 2003; Walker et al 2002). With the exception of DCS, the locus of action in the brain of these drugs is not yet known. On the basis of previous work, however, modulation of dopaminergic and noradrenergic systems in the rat mPFC is likely to modulate the rate of extinction (McCormick and Thompson 1982; Morrow et al 1999). DCS has been shown to be effective when infused into the BLA (Walker et al 2002), and clinical studies show that administering DCS to acrophobic subjects who are undergoing exposure therapy improves the effectiveness of the therapy (Ressler et al 2004). However, there are some limitations to this approach, such as CS nonspecificity and tolerance to repeated DCS (Ledgerwood et al 2005; Parnas et al 2005). An alternative approach would be to use the metabolic enhancer MB (Riha et al 2005). A metabolic approach differs from the transmitter-receptor approach because it is not selective for a single transmitter system or brain region but targets all the synapses that require increased energy during postextinction memory consolidation, such as in the various prefrontal cortex regions (Gonzalez-Lima and Bruchey, 2004). Other approaches to activating mPFC during exposure therapy could include repetitive transcranial magnetic stimulation (Cohen et al 2004), deep brain stimulation (Abelson et al 2005), or even meditation (Lazar et al 2000). Thus, prefrontal activation achieved pharmacologically, physiologically, or psychologically could serve as a useful adjunct to exposure therapy by strengthening memory for the extinction (safety) experience.

This work was supported by NIH grants: R01-MH58883, S06-GM08239, and R21-MH072156 (to GJQ), the Philippe Foundation (to RG), and NIH grant R01-NS37755 (to FGL).

Aspects of this work were presented at the conference "Extinction: The Neural Mechanisms of Behavior Change", February 2–6, 2005 in Ponce, Puerto Rico. The Conference was sponsored by the National Institute of Mental Health, National Institute on Drug Abuse, Ponce School of Medicine, University of Puerto Rico Centers of Biomedical Research Excellence (COBRE) Program, Pfizer Global Pharmaceutical, and the Municipality of Ponce.

Abelson JL, Curtis GC, Sagher O, Albucher RC, Harrigan M, Taylor SF, et al (2005): Deep brain stimulation for refractory obsessive-compulsive disorder. *Biol Psychiatry* 57:510–516.

Anderson P, Jacobs C, Rothbaum BO (2004): Computer-supported cognitive behavioral treatment of anxiety disorders. *J Clin Psychol* 60:253–267.

Anglada-Figueroa D, Quirk GJ (2005): Lesions of the basal amygdala block expression of conditioned fear, but not extinction. *J Neurosci* 25:9680–9685.

Baeg EH, Kim YB, Jang J, Kim HT, Mook-Jung I, Jung MW (2001): Fast spiking and regular spiking neural correlates of fear conditioning in the medial prefrontal cortex of the rat. *Cereb Cortex* 11:441–451.

Baker JD, Azorlosa JL (1996): The NMDA antagonist MK-801 blocks the extinction of Pavlovian fear conditioning. *Behav Neurosci* 110:618–620.

Barrett D, Shumake J, Jones D, Gonzalez-Lima F (2003): Metabolic mapping of mouse brain activity after extinction of a conditioned emotional response. *J Neurosci* 23:5740–5749.

Berretta S, Pantazopoulos H, Caldera M, Pantazopoulos P, Paré D (2005): Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. *Neuroscience* 132:943–953.

Bouton ME (2002): Context, ambiguity, and unlearning: Sources of relapse after behavioral extinction. *Biol Psychiatry* 52:976–986.

Bremner JD (2002): Neuroimaging studies in post-traumatic stress disorder. *Curr Psychiatry Rep* 4:254–263.

Bremner JD (2003): Functional neuroanatomical correlates of traumatic stress revisited 7 years later, this time with data. *Psychopharmacol Bull* 37:6–25.

Burgos-Robles A, Santini E, Quirk GJ (2004): Blockade of NMDA receptors in the medial prefrontal cortex impairs consolidation of fear extinction. *Soc Neurosci Abstr* Program No. 328.14.

Butter CM, Mishkin M, Rosvold HE (1963): Conditioning and extinction of a food-rewarded response after selective ablation of frontal cortex in rhesus monkeys. *Exp Neurol* 7:65–75.

Cain CK, Blouin AM, Barad M (2004): Adrenergic transmission facilitates extinction of conditional fear in mice. *Learn Mem* 11:179–187.

Callaway NL, Riha PD, Wrubel KM, McCollum D, Gonzalez-Lima F (2002): Methylene blue restores spatial memory retention impaired by an inhibitor of cytochrome oxidase in rats. *Neurosci Lett* 332:83–86.

Cassell MD, Wright DJ (1986): Topography of projections from the medial prefrontal cortex to the amygdala in the rat. *Brain Res Bull* 17:321–333.

Chan KH, Morell JR, Jarrard LE, Davidson TL (2001): Reconsideration of the role of the hippocampus in learned inhibition. *Behav Brain Res* 119:111–130.

Charney DS (2004): Psychobiological mechanisms of resilience and vulnerability: Implications for successful adaptation to extreme stress. *Am J Psychiatry* 161:195–216.

Charney DS, Deutch AY, Krystal JH, Southwick SM, Davis M (1993): Psychobiologic mechanisms of posttraumatic stress disorder. *Arch Gen Psychiatry* 50:294–305.

Chhatwal JP, Davis M, Maguschak KA, Ressler KJ (2005): Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. *Neuropsychopharmacology* 30:516–524.

Cohen H, Kaplan Z, Kotler M, Kouperman I, Moisa R, Grisaru N (2004): Repetitive transcranial magnetic stimulation of the right dorsolateral prefrontal cortex in posttraumatic stress disorder: A double-blind, placebo-controlled study. *Am J Psychiatry* 161:515–524.

Condé F, Maire-Lepoivre E, Audinat E, Crepel F (1995): Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. *J Comp Neurol* 352:567–593.

Corcoran KA, Maren S (2003): Reversible inactivation of medial prefrontal cortex disrupts the retrieval of fear extinction. *Soc Neurosci Abstr* 199.8.

Cox J, Westbrook RF (1994): The NMDA receptor antagonist MK-801 blocks acquisition and extinction of conditioned hypoalgesic responses in the rat. *Q J Exp Psychol B* 47:187–210.

Falls WA, Miserendino MJ, Davis M (1992): Extinction of fear-potentiated startle: Blockade by infusion of an NMDA antagonist into the amygdala. *J Neurosci* 12:854–863.

Farinelli M, Deschaux O, Hugues S, Thevenet A, Garcia R (in press): Hippocampal train stimulation modulates recall of fear extinction independently of prefrontal cortex synaptic plasticity and lesions. *Learn Mem*.

Floyd NS, Price JL, Ferry AT, Keay KA, Bandler R (2001): Orbitomedial prefrontal cortical projections to hypothalamus in the rat. *J Comp Neurol* 432:307–328.

Garcia R (2002): Stress, synaptic plasticity, and psychopathology. *Rev Neurosci* 13:195–208.

Gewirtz JC, Falls WA, Davis M (1997): Normal conditioned inhibition and extinction of freezing and fear-potentiated startle following electrolytic lesions of medical prefrontal cortex in rats. *Behav Neurosci* 111:712–726.

Gilboa A, Shalev AY, Laor L, Lester H, Louzoun Y, Chisin R, et al (2004): Functional connectivity of the prefrontal cortex and the amygdala in posttraumatic stress disorder. *Biol Psychiatry* 55:263–272.

Gonzalez-Lima F, Bruchey AK (2004): Extinction memory improvement by the metabolic enhancer methylene blue. *Learn Mem* 11:633–640.

Gottfried JA, Dolan RJ (2004): Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. *Nat Neurosci* 7:1144–1152.

Hermans D, Dirikx T, Vansteenwegenin D, Baeyens F, Van den Bergh O, Eelen P (2005): Reinstatement of fear responses in human aversive conditioning. *Behav Res Ther* 43:533–551.

Herry C, Garcia R (2002): Prefrontal cortex long-term potentiation, but not long-term depression, is associated with the maintenance of extinction of learned fear in mice. *J Neurosci* 22:577–583.

Herry C, Garcia R (2003): Behavioral and paired-pulse facilitation analyses of long-lasting depression at excitatory synapses in the medial prefrontal cortex in mice. *Behav Brain Res* 146:89–96.

Herry C, Vouimba RM, Garcia R (1999): Plasticity in the mediodorsal thalamo-prefrontal cortical transmission in behaving mice. *J Neurophysiol* 82: 2827–2832.

Hugues S, Chessel A, Léna I, Marsault R, Garcia R (in press): Prefrontal infusion of PD098059 immediately after fear extinction training blocks extinction-associated prefrontal synaptic plasticity and decreases prefrontal ERK2 phosphorylation. *Synapse*.

Hugues S, Deschaux O, Garcia R (2004): Postextinction infusion of a mitogen-activated protein kinase inhibitor into the medial prefrontal cortex impairs memory of the extinction of conditioned fear. *Learn Mem* 11:540–543.

Hurley KM, Herbert H, Moga MM, Saper CB (1991): Efferent projections of the infralimbic cortex of the rat. *J Comp Neurol* 308:249–276.

Jay TM, Burette F, Laroche S (1995): NMDA receptor-dependent long-term potentiation in the hippocampal afferent fibre system to the prefrontal cortex in the rat. *Eur J Neurosci* 7:247–250.

Jay TM, Witter MP (1991): Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. *J Comp Neurol* 313:574–586.

Kaczmarek L (2002): c-Fos in learning: Beyond the mapping of neuronal activity. In: Kaczmarek L, Robertson HA, editors. *Handbook of Chemical Neuroanatomy: Immediate Early Genes and Inducible Transcription Factors in Mapping of the Central Nervous System Function and Dysfunction*. Amsterdam: Elsevier, 189–215.

Kandel ER (2001): The molecular biology of memory storage: A dialogue between genes and synapses. *Science* 294:1030–1038.

Kimble DP, Kimble RJ (1970): The effect of hippocampal lesions on extinction and "hypothesis" behavior in rats. *Physiol Behav* 5:735–738.

Konorski J (1967): *Integrative Activity of the Brain*. Chicago: University of Chicago Press.

Laviolette SR, Lipski WJ, Grace AA (2005): A subpopulation of neurons in the medial prefrontal cortex encodes emotional learning with burst and frequency codes through a dopamine D4 receptor-dependent basolateral amygdala input. *J Neurosci* 25:6066–6075.

Lazar SW, Bush G, Gollub RL, Fricchione GL, Khalsa G, Benson H (2000): Functional brain mapping of the relaxation response and meditation. *Neuroreport* 11:1581–1585.

Lebron K, Milad MR, Quirk GJ (2004): Delayed recall of fear extinction in rats with lesions of ventral medial prefrontal cortex. *Learn Mem* 11:544–548.

Ledgerwood L, Richardson R, Cranney J (2003): Effects of D-cycloserine on extinction of conditioned freezing. *Behav Neurosci* 117:341–349.

Ledgerwood L, Richardson R, Cranney J (2005): D-cycloserine facilitates extinction of learned fear: Effects on reacquisition and generalized extinction. *Biol Psychiatry* 57:841–847.

Likhtik E, Pelletier JG, Paz R, Paré D (2005): Prefrontal control of the amygdala. *J Neurosci* 25:7429–7437.

Lin CH, Yeh SH, Lu HY, Gean PW (2003): The similarities and diversities of signal pathways leading to consolidation of conditioning and consolidation of extinction of fear memory. *J Neurosci* 23:8310–8317.

Lissek S, Powers AS, McClure EB, Phelps EA, Woldehawariat G, Grillon C, et al (2005): Classical fear conditioning in the anxiety disorders: A meta-analysis. *Behav Res Ther* 43:1391–1424.

Lu KT, Walker DL, Davis M (2001): Mitogen-activated protein kinase cascade in the basolateral nucleus of amygdala is involved in extinction of fear-potentiated startle. *J Neurosci* 21:RC162.

Maroun M, Richter-Levin G (2003): Exposure to acute stress blocks the induction of long-term potentiation of the amygdala-prefrontal cortex pathway in vivo. *J Neurosci* 23:4406–4409.

Martinez JL Jr, Jensen RA, Vasquez BJ, McGuinness T, McGaugh JL (1978): Methylene blue alters retention of inhibitory avoidance responses. *Physiol Psychol* 6:387–390.

McCormick DA, Thompson RF (1982): Locus coeruleus lesions and resistance to extinction of a classically conditioned response: Involvement of the neocortex and hippocampus. *Brain Res* 245:239–249.

McDonald AJ (1991): Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. *Neuroscience* 44:1–14.

McDonald AJ, Mascagni F, Guo L (1996): Projections of the medial and lateral prefrontal cortices to the amygdala: A Phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience* 71:55–75.

Mickley GA, Kenmuir CL, Yocom AM, Wellman JA, Biada JM (2005): A role for prefrontal cortex in the extinction of a conditioned taste aversion. *Brain Res* 1051:176–182.

Milad MR, Quinn BT, Pitman RK, Orr SP, Fischl B, Rauch SL (2005): Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. *Proc Natl Acad Sci U S A* 102:10706–10711.

Milad MR, Quirk GJ (2002): Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature* 420:70–74.

Milad MR, Rauch SL, Pitman RK, Quirk GJ (in press): Fear extinction in rats: Implications for human brain imaging and anxiety disorders. *Biol Psychol*

Milad MR, Vidal-Gonzalez I, Quirk GJ (2004): Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. *Behav Neurosci* 118:389–394.

Morgan MA, LeDoux JE (1995): Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behav Neurosci* 109:681–688.

Morgan MA, Romanski LM, LeDoux JE (1993): Extinction of emotional learning: Contribution of medial prefrontal cortex. *Neurosci Lett* 163:109–113.

Morgan MA, Schulkin J, LeDoux JE (2003): Ventral medial prefrontal cortex and emotional perseveration: The memory for prior extinction training. *Behav Brain Res* 146:121–130.

Morrow BA, Elsworth JD, Rasmusson AM, Roth RH (1999): The role of mesoprefrontal dopamine neurons in the acquisition and expression of conditioned fear in the rat. *Neuroscience* 92:553–564.

Myers KM, Davis M (2002): Behavioral and neural analysis of extinction. *Neuron* 36:567–584.

Myers KM, Davis M (2004): Temporary inactivation of the infralimbic region of medial prefrontal cortex via localized infusion of muscimol or baclofen does not impair conditioned inhibition or extinction of fear potentiated startle in rats. *Soc Neurosci Abstr* 328.2.

Naylor GJ, Martin B, Hopwood SE, Watson Y (1986): A two-year double-blind crossover trial of the prophylactic effect of methylene blue in manic-depressive psychosis. *Biol Psychiatry* 21:915–920.

Paré D, Smith Y (1993a): Distribution of GABA immunoreactivity in the amygdaloid complex of the cat. *Neuroscience* 57:1061–1076.

Paré D, Smith Y (1993b): The intercalated cell masses project to the central and medial nuclei of the amygdala in cats. *Neuroscience* 57:1077–1090.

Parnas AS, Weber M, Richardson R (2005): Effects of multiple exposures to D-cycloserine on extinction of conditioned fear in rats. *Neurobiol Learn Mem* 83:224–231.

Pavlov I (1927): *Conditioned Reflexes*. London: Oxford University Press.

Peri T, Ben Shakhar G, Orr SP, Shalev AY (2000): Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. *Biol Psychiatry* 47:512–519.

Phelps EA, Delgado MR, Nearing KI, LeDoux JE (2004): Extinction learning in humans: Role of the amygdala and vmPFC. *Neuron* 43:897–905.

Pirot S, Jay TM, Glowinski J, Thierry AM (1994): Anatomical and electrophysiological evidence for an excitatory amino acid pathway from the thalamic mediodorsal nucleus to the prefrontal cortex in the rat. *Eur J Neurosci* 6:1225–1234.

Ponnusamy R, Nissim HA, Barad M (2005): Systemic blockade of D2-like dopamine receptors facilitates extinction of conditioned fear in mice. *Learn Mem* 12:399–406.

Quirk GJ (2002): Memory for extinction of conditioned fear is long-lasting and persists following spontaneous recovery. *Learn Mem* 9:402–407.

Quirk GJ, Likhtik E, Pelletier JG, Paré D (2003): Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. *J Neurosci* 23:8800–8807.

Quirk GJ, Russo GK, Barron JL, Lebron K (2000): The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *J Neurosci* 20: 6225–6231.

Rauch SL, Shin LM, Phelps EA (2006): Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research past, present and future. *Biol Psychiatry* 60:376–382.

Rauch SL, Shin LM, Segal E, Pitman RK, Carson MA, McMullin K, et al (2003): Selectively reduced regional cortical volumes in post-traumatic stress disorder. *Neuroreport* 14:913–916.

Rescorla RA (2004): Spontaneous recovery. *Learn Mem* 11:501–509.

Rescorla RA, Heth CD (1975): Reinstatement of fear to an extinguished conditioned stimulus. *J Exp Psychol Anim Behav Process* 1:88–96.

Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, et al (2004): Cognitive enhancers as adjuncts to psychotherapy: Use of D-cycloserine in phobic individuals to facilitate extinction of fear. *Arch Gen Psychiatry* 61:1136–1144.

Rhodes SE, Killcross S (2004): Lesions of rat infralimbic cortex enhance recovery and reinstatement of an appetitive Pavlovian response. *Learn Mem* 11:611–616.

Riha PD, Bruchey AK, Echevarria DJ, Gonzalez-Lima F (2005): Memory facilitation by methylene blue: Dose-dependent effect on behavior and brain oxygen consumption. *Eur J Pharmacol* 511:151–158.

Royer S, Martina M, Paré D (1999): An inhibitory interface gates impulse traffic between the input and output stations of the amygdala. *J Neurosci* 19:10575–10583.

Royer S, Paré D (2002): Bidirectional synaptic plasticity in intercalated amygdala neurons and the extinction of conditioned fear responses. *Neuroscience* 115:455–462.

Santini E, Ge H, Ren K, Pena DO, Quirk GJ (2004): Consolidation of fear extinction requires protein synthesis in the medial prefrontal cortex. *J Neurosci* 24:5704–5710.

Santini E, Muller RU, Quirk GJ (2001): Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent memory. *J Neurosci* 21:9009–9017.

Shin LM, Orr SP, Carson MA, Rauch SL, Macklin ML, Lasko NB, et al (2004): Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female Vietnam veterans with PTSD. *Arch Gen Psychiatry* 61:168–176.

Shin LM, Whalen PJ, Pitman RK, Bush G, Macklin ML, Lasko NB, et al (2001): An fMRI study of anterior cingulate function in posttraumatic stress disorder. *Biol Psychiatry* 50:932–942.

Shumake J, Barrett D, Gonzalez-Lima F (2005): Behavioral characteristics of rats predisposed to learned helplessness: Reduced reward sensitivity, increased novelty seeking, and persistent fear memories. *Behav Brain Res* 164:222–230.

Shumake J, Poremba A, Edwards E, Gonzalez-Lima F (2000): Congenital helpless rats as a genetic model for cortex metabolism in depression. *Neuroreport* 11:3793–3798.

Sierra-Mercado D, Burgos-Robles A, Corcoran KA, Lebron K, Quirk GJ (2005): Inactivation of the ventromedial prefrontal cortex reduces the expression of conditioned fear. *Soc Neurosci Abstr* 649.9.

Sokoloff L (1992): Imaging techniques in studies of neural functions. In: Gonzalez-Lima F, Finkenstadt T, Scheich H, editors. *Advances in Metabolic Mapping Techniques for Brain Imaging of Behavior and Learning Functions*. Boston: Kluwer, 1–37.

Song E, Kim JJ (2004): Effects of auditory cortex lesions on acquisition and extinction of fear conditioning to two different auditory stimuli in rats. *Soc Neurosci Abstr* 773.16.

Sotres-Bayon F, Bush DE, LeDoux JE (2004): Emotional perseveration: An update on prefrontal-amygdala interactions in fear extinction. *Learn Mem* 11:525–535.

Sotres-Bayon F, Cain CK, LeDoux JE (2006): Brain mechanisms of fear extinction: Historical perspectives on the contribution of prefrontal cortex. *Biol Psychiatry* 60:329–336.

Teich AH, McCabe PM, Gentile CC, Schneiderman LS, Winters RW, Liskowsky DR, et al (1989): Auditory cortex lesions prevent the extinction of Pavlovian differential heart rate conditioning to tonal stimuli in rabbits. *Brain Res* 480:210–218.

van Minnen A, Hagenaars M (2002): Fear activation and habituation patterns as early process predictors of response to prolonged exposure treatment in PTSD. *J Trauma Stress* 15:359–367.

Vertes RP (2004): Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse* 51:32–58.

Vouimba RM, Garcia R, Baudry M, Thompson RF (2000): Potentiation of conditioned freezing following dorsomedial prefrontal cortex lesions does not interfere with fear reduction in mice. *Behav Neurosci* 114:720–724.

Walker DL, Davis M (2002): The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. *Pharmacol Biochem Behav* 71:379–392.

Walker DL, Ressler KJ, Lu KT, Davis M (2002): Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. *J Neurosci* 22:2343–2351.

Weible AP, McEchron MD, Disterhoft JF (2000): Cortical involvement in acquisition and extinction of trace eyeblink conditioning. *Behav Neurosci* 114:1058–1067.