The Role of the Amygdala in the Extinction of Conditioned Fear

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The amygdala has long been known to play a central role in the acquisition and expression of fear. More recently, convergent evidence has implicated the amygdala in the extinction of fear as well. In rodents, some of this evidence comes from the infusion of drugs directly into the amygdala and, in particular, into the basolateral complex of the amygdala, during or after extinction learning. In vivo electrophysiology has identified cellular correlates of extinction learning and memory in the lateral nucleus of that structure. Human imaging experiments also indicate that amygdaloid activity correlates with extinction training. In addition, some studies have directly identified changes in molecular constituents of the basolateral amygdala. Together these experiments strongly indicate that the basolateral amygdala plays a crucial role in extinction learning. Interpreted in the light of these findings, several recent in vitro electrophysiology studies in amygdala-containing brain slices are suggestive of potential synaptic and circuit bases of extinction learning.

Key Words: Fear, extinction, amygdala, endocannabinoid, calcineurin, L-type voltage-gated calcium channels

Extinction of conditioned fear is crucial both as a paradigm of inhibitory learning and as a model of behavior therapy for human anxiety disorder. As such it offers an excellent opportunity to identify the fundamental mechanisms of inhibitory learning and to use such fundamental science to improve psychiatric treatment by new methods. An important step in the understanding of such basic mechanisms is to identify the anatomical substrates of extinction learning. The amygdala, particularly the basolateral amygdaloid complex (BLA), is one location that seems to play an important role in fear extinction learning and expression.

It has long been known that the acquisition and expression of fear depend on the amygdala. Among the earliest evidence for this was the description of the Kluver-Bucy syndrome in monkeys, who lost all fear (as well as becoming hyperphagic and hypersexual) after anterior temporal lobectomy (Kluver and Bucy 1939). Since those initial studies, many more have confirmed the crucial role that the amygdala plays in the acquisition and expression of fear and refined the roles of different nuclei within that structure in fear learning.

In Pavlovian fear conditioning, a novel stimulus that is initially neutral for the animal, usually a tone or a light, serves as the conditioned stimulus (CS). When this stimulus is temporally paired with an intrinsically aversive stimulus, such as a footshock (the unconditioned stimulus [US]), the CS becomes a cue for a conditioned fear response. This response includes most but not all of the responses to the US itself, such as increases of heart rate and blood pressure, changes in respiration, and behavioral measures such as behavioral freezing or fear potentiation of startle (Davis 1992b). Experiments using assays like these combined with lesions or pharmacological inactivation of the amygdala have revealed that the central nucleus sends the output of the amygdala to various cortical and brain stem structures that mediate these different aspects of the fear response (Davis 1992a). In contrast, signals from the stimuli that lead to fear conditioning (i.e., from the CS and US) converge in the basolateral complex of the amygdala, which processes those stimuli and sends its output to the central nucleus (Romanski et al 1993; Shi and Davis 1999). Thus, the basolateral complex of the amygdala seems to be a crucial structure in fear learning.

The extinction of fear and extinction in general is considered to be inhibitory learning, which prevents the expression of an intact association rather than erasing it. Because some of the data reviewed here might bring this concept into doubt, it is worthwhile to review the evidence for the view of extinction as inhibitory learning. The proposal that extinction is inhibitory learning was originally made by Pavlov himself, on the basis of his experiments with conditioned salivation in dogs (Pavlov 1927). The evidence for the preservation of the original association in fear extinction comes from situations in which the conditioned response (for our purposes, the fear response) returns after extinction, with no further training by pairing the CS with the US. The most obvious of these is spontaneous recovery, which describes the return of fear of CS with time after extinction (Baum 1988). In most experiments, the most rapid recovery comes between the end of extinction training and the final test, often 1 day later, when about 30% of the original response returns, even after twice as much extinction training than is needed to bring responding during extinction to zero. In addition, the fear response to the CS can be "reinstated" by presentations of US alone (Rescorla and Heth 1975) or other stressful stimuli without paired CS presentations. Perhaps the most convincing evidence for extinction being inhibitory learning is "renewal," in which fear of the CS returns when the CS is presented in a context different from that of extinction itself (Bouton and King 1983). In fact, mice can be moved back and forth from the extinction context to another, expressing extinction in the extinction context and nearly intact fear in the other (Tim Bredy and MB, unpublished results). This extensive psychological evidence argues that extinction is new learning superimposed on an intact fear association, but the nature of that learning remains uncertain both at the neurobiological and behavioral levels, because the psychological data do not differentiate between an inhibitory association, an excitatory association with an opposing motivational state, or an inhibition at the level of the CS. Despite this evidence of new learning, which leaves the original association intact, recent physiological and molecular studies from one of the authors (PWG, reviewed here) have raised new questions about this central tenet of extinction theory, suggesting that extinction might represent erasure. Rec-

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Received August 29, 2005; revised March 28, 2006; accepted May 12, 2006.

0006-3223/06/$32.00
doi:10.1016/j.biopsych.2006.05.029

onciling these findings will certainly be an important project in extinction research over the next few years.

Evidence for Amygdaloid Involvement in Fear Extinction

There are several ways to localize the control of a behavior to a specific brain location. These include lesion studies, in vivo electrophysiology studies, local infusion studies, biochemical or immunohistochemical studies of changes in molecules that correlate to behavioral changes, and studies of physiological correlates to behavior in slices of the target region in brain after behavioral training.

Electrical or neurotoxic lesions of candidate regions have been the classical approach to demonstrating that a region is necessary to generate a behavior. Unfortunately, irreversible lesions of the amygdala are not useful for the study of fear extinction, because the amygdala is required for the expression of fear itself. Clearly, it is impossible to study the extinction of fear that is no longer expressed. Despite the absence of lesion studies, substantial evidence is accumulating to indicate that BLA and perhaps specifically the lateral nucleus (LA) of the amygdala play a crucial role in extinction learning. These data come from a combination of infusion, in vivo electrophysiology, and correlative molecular studies. Notably, the electrophysiological data are strongest for LA, the first point of convergence in the amygdala for CS and US input (Romanski et al 1993), whereas the infusion studies, because of the difficulties of localization, really only implicate BLA as a whole.

N-methyl-D-aspartate Receptors and L-type Voltage-Gated Calcium Channels in BLA

The first data implicating the BLA in extinction came from infusion of the N-methyl-D-aspartate (NMDA) antagonist, 2-amino-5-phosphonopentanoic acid (APV), into that structure through chronically implanted cannulae in rats (Falls et al 1992). These researchers used stereotaxic surgery to implant cannulae directed at basolateral amygdala on both sides, allowed the animals to recover, and then fear-conditioned the animals with 10 light CS-shock US pairings. Measuring fear with the fear-potentiated startle (FPS) paradigm, these researchers established a pre-extinction baseline and then, on the next 2 days, infused vehicle or different doses of APV bilaterally before 30 CS-alone presentations. One day after extinction training, the rats were again tested for FPS, drug-free, and showed a dose-dependent blockade of extinction in APV-treated groups. This experiment showed that extinction learning was dependent on NMDA receptors in BLA. In a recent follow-up to this study, intra-BLA infusion of d-cycloserine, an agonist at the glycine binding site of the NMDA receptor, facilitated suboptimal extinction in a dose-dependent manner (Walker et al 2002). The same effect was seen with systemic injections of d-cycloserine, predicting the potential utility of this drug as an adjunct to behavior therapy of human anxiety disorders, which is modeled on fear extinction. In fact, this promise has already been realized; d-cycloserine administration once before each of two weekly therapy sessions was recently shown to accelerate significantly a virtual-reality–based behavior therapy of human acrophobic patients (Ressler et al 2004).

There are at least two caveats to the interpretation of these infusions of drugs targeting the NMDA receptor. The first is an objection raised to all infusion experiments. It is impossible to guarantee that a drug is affecting only or even mostly a specific anatomical target. For example, in one experiment, an infusion of a protein synthesis inhibitor (anisomycin) into basolateral amygdala of rats blocked protein synthesis in a wide swath of structures, including all the nuclei of the amygdala and the overlying striatum (Maren et al 2003). However, it has been possible to distinguish the behavioral effects of infusions of a glucocorticoid receptor agonist and an antagonist directed at central or basolateral nuclei of the amygdala in rats (Roozendaal and McGaugh 1997) and, even more remarkably, the effects of lidocaine infusions into LA or the basolateral nucleus in mice (Calandreau et al 2005).

The second caveat is really just a clarification. Extinction was not measured "on line" during the FPS studies cited previously. The presentations of the CS to generate extinction were not accompanied by tests of FPS, which were only performed before and 1 day after extinction training (Falls et al 1992). Thus it cannot be determined whether the NMDA antagonist had its effects immediately, during the acute phase of extinction learning, or later, during the consolidation of extinction learning. In fact, in later experiments, systemic administration of the NMDA inhibitor, CPP, had no effect on extinction of conditioned freezing as a measure of fear, measured "on line," during the CS presentations (Santini et al 2001). Nevertheless, consistent with the previous results, when fear was measured the next day, the CPP-treated rats showed no memory of their extinction training compared with vehicle-injected control subjects. The CPP thus blocked long-term but not short-term extinction. This finding implicated BLA NMDA receptors in the consolidation of extinction learning but not in its induction.

It has since been found that L-type voltage-gated calcium channels (LVGCCs) are necessary for the acute phase of extinction but not for the acquisition or expression of conditioned freezing in mice (Cain et al 2002). Consistent with these results obtained with systemic drug treatments, direct infusion of the LVGCC antagonist, nimodipine, into BLA of mice also causes a dose-dependent blockade of both acute and long-term extinction learning, whereas infusion of the LVGCC agonist, BayK8644, causes a dose-dependent facilitation of extinction (CK Cain, S Jami, and MB, unpublished results).

Endocannabinoids

The endocannabinoid system (Piomelli 2003)—which comprises the endocannabinoids, lipid signaling molecules, and their endogenous receptors, the cannabinoid receptors—has recently emerged as an important player in the regulation of emotionality (for review, see Wotjak 2005), including fear extinction learning (Marsicano et al 2002). Cannabinoid receptor type 1 (CB1 receptor)-deficient mice were strongly impaired in both short-term and long-term extinction of cue-conditioned fear (Marsicano et al 2002). Importantly, the initial acquisition of fear during CS-US pairings as well as the subsequent consolidation process were unchanged in CB1 receptor-deficient mice compared with wild-type littermate control subjects. This phenotype was mimicked by systemic injections of the specific CB1 receptor antagonist, SR141716, either before conditioning or before the extinction trial. As with the knock-out mice, only extinction was impaired in this pharmacological experiment, whereas the acquisition and consolidation of fear memory were unaffected. This result strongly indicates that the endocannabinoid system is activated during the extinction trial and is required specifically during that period of time. Indeed, elevated endocannabinoid levels were measured during the extinction trial in BLA. This

observation, together with the presence of CB1 receptor messenger RNA (mRNA) (Marsicano and Lutz 1999) and protein (Katona et al 2001; McDonald and Mascagni 2001) in BLA, strongly suggests that the amygdala plays a crucial role in the process of CB1 receptor-mediated fear extinction. However, other brain regions including the prefrontal cortex express CB1 receptors in projecting neurons that innervate the amygdala and thus might also play important roles.

These findings have clinical promise as prolongation of the activity of the endocannabinoid system during extinction training by systemic inhibition of endocannabinoid breakdown and re-uptake with AM404 increases the rate of extinction of FPS (Chhatwal et al 2005a).

It is noteworthy that systemic injection of mice with the CB1 receptor antagonist, SR141716, also strongly impairs extinction of conditioned fear to context, a task dependent on both the hippocampus and amygdala (Suzuki et al 2004). In contrast, the endocannabinoid system does not seem to be involved in the extinction of an appetitive operant task (Holter et al 2005), suggesting that the endocannabinoid system is selectively involved in protocols involving fear, stress, and/or anxiety. At present, studies are in progress to define whether associative and/or non-associative components of fear conditioning (Kamprath and Wotjak 2004) depend on CB1 receptors for their extinction. Cannabinoid receptor type 1–deficient mice are also impaired in the extinction of spatial memory (Varvel and Lichtman 2002).

The mechanisms underlying the role of CB1 receptors in fear extinction have not yet been unraveled. In the lateral and basolateral amygdala, CB1 receptors are expressed in a population of cholecystokinin (CCK)-positive interneurons (Katona et al 2001; Marsicano and Lutz 1999), but they are also present in glutamatergic neurons of this brain region (Marsicano and Lutz 1999). Consistent with this dual expression, CB1 receptor agonists repress both γ-aminobutyric acid (GABA)-ergic and glutamatergic neurotransmission in the lateral amygdala (LA) in response to stimulation from the external capsule (Azad et al 2003). Recent investigations with conditional CB1 receptor-deficient mice that lack CB1 receptor expression either in all GABAergic forebrain neurons or in all principal projecting forebrain neurons have suggested that both neuronal subpopulations are required for proper extinction of fear memories (G. Marsicano, K. Kamprath, C. Wotjak, and BL, unpublished results). Some new work also points to potential second messengers involved in the endocannabinoid effects in extinction. When tested after CS-alone extinction training (compared with no CS control subjects), CB1 receptor-deficient mice failed to show the increase of mitogen-activated protein (MAP) kinase phosphorylation and calcineurin protein in BLA shown by wild-type littermates (Cannich et al 2004). As discussed in the following section, these biochemical changes have been shown to be required for both fear extinction and depotentiation (see subsequent discussion and Lin et al 2003a, 2003b, 2003c; Lu et al 2001). Although these data suggest strongly that endocannabinoids and CB1 receptor play an important role in the basolateral amygdala in the mediation of extinction learning, the exact mechanism of their contribution remains unknown, as it does to date for all the molecules identified so far as essential to extinction.

Does Fear Extinction Involve Erasure of Changes That Occur With Fear Conditioning?

A series of experiments combining basolateral amygdala infusions, electrophysiology, and correlative molecular studies of BLA tissue has added substantially to the data supporting a role for the BLA in extinction learning (Lin et al 2003, 2003b, 2003c). In addition to providing substantial support for the role of the BLA in extinction learning, these experiments provide strong evidence that extinction does involve reversal of at least some of the changes that underlie the original associative fear learning. The majority of investigators in the field accept the idea that extinction does not affect the original fear memory, but rather that it represents an independent separate inhibitory learning, on the basis of the kind of evidence cited in the introduction to this article. That is, the memory trace of fear conditioning must remain intact through extinction, given the return of fear without further pairing of CS-US through spontaneous recovery, reinstatement, and renewal. Nevertheless, Po-Wu Gean and his colleagues have accumulated substantial evidence arguing that extinction represents, at least in part, a reversal or erasure of the synaptic changes that underlie conditioned fear itself.

In one group of experiments, this group examined depotentiation in the amygdala (i.e., the reversal of long-term potentiation [LTP], a form of synaptic strengthening) (Lin et al 2003a). Substantial evidence indicates that long-term LTP in the amygdala may make an important contribution to the acquisition and expression of conditioned fear (Bauer et al 2001; Blair et al 2001; Maren 1999; Rogan et al 1997). Depotentiation is a method for reversal of LTP by administering low-frequency stimulation to the same synapse shortly after the induction of LTP by high-frequency stimulation (Bashir and Collingridge 1994; Fujii et al 1991). Gean and his colleagues demonstrated LTP and depotentiation, in vivo, in a pathway from the external capsule to the LA and showed that the depotentiation could be blocked by antagonists of the NMDA receptor, of LVGCCs, and of calcineurin (protein phosphatase 2, a molecule previously associated with constraints on LTP and learning) (Malleret et al 2001). Furthermore, they correlated depotentiation with decreases in the phosphorylation of Akt (a substrate of phosphotidylinositol 3-[PI-3] kinase), which they had previously shown to be essential for long-term fear conditioning and for LTP (Lin et al 2001). They also showed decreases in MAP kinase phosphorylation and increases in calcineurin activity in amygdala samples after depotentiation. Finally, they showed that low-frequency stimulation administered in vivo 10 min after fear conditioning, could also decrease ("quench") FPS. Thus, synaptic depotentiation correlates well with a decrease in behavioral response. However, although these experiments are quite convincing about the correlation, the data also suggest that depotentiation, or quenching, might not end up contributing to most extinction learning. Both depotentiation and quenching require that low-frequency stimulation be administered within minutes of LTP or fear induction, a constraint that certainly does not apply to extinction of conditioned fear or to the decrease in LTP measured in the LA after extinction (Rogan et al 1997).

In later experiments, the Gean group used a more typical behavioral extinction protocol, in which they trained animals to fear a light (assayed by FPS) and then extinguished that fear 24 hours later by repeated presentation of that light, resulting in a significant decrease of potentiated startle (Lin et al 2003b, 2003c). In these experiments, they demonstrated that some of the same molecules involved in depotentiation were also involved in extinction, by either blocking extinction with antagonists or by biochemical correlation. These common molecules include calcineurin, NMDA receptors, MAP kinase, and PI-3 kinase. In addition, they demonstrated a dependence of extinction on translation (protein synthesis from mRNAs) but not on transcrip-

tion (mRNA synthesis from DNA templates), although the testing might have been done too soon (10 min) after extinction training to detect a role for transcription. Although there are some discrepancies in these findings with those of other groups studying extinction, particularly in showing the early dependence of extinction on NMDA receptor activity (see Santini et al 2001), they make an intriguing case that at least some of the synaptic changes underlying fear conditioning are reversed by extinction training. Although the Gean group did not directly test LTP in their extinction experiments, the authors report strong parallels between depotentiation, that is reversal of LTP on a brief time scale (min), and extinction (behavioral reversal of fear measured at 24 hours). Writing that "extinction training might weaken or erase the original memory (p. 8315)," they hypothesize that both the behavioral profiles and the correlated changes in protein phosphorylation indicate that a similar reversal in LTP is involved in both quenching and extinction, despite the differences in time scale.

However, there might be other explanations of these observations. The authors suggest that although the memory might be erased in the amygdala, it might be conserved in another area, for example, prefrontal cortex. This kind of two track system, where the memory is erased in one location but preserved in another, has been proposed for extinction of conditioned eyeblink (Mauk et al 2004; Medina et al 2001). In theory, the two locations need not be in different brain nuclei; such a system would work even were the erased and conserved sites were within a circuit in a single brain location. In this regard, it is interesting that two populations of cells have been characterized in the dorsal subnucleus of the LA, one of whose activity is transiently increased after fear conditioning, whereas the responses of the others to the CS remains elevated even through extinction (Repa et al 2001).

Most recently, Gean et al have looked at the effect of fear conditioning and extinction on the surface expression of α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA)-type glutamate receptors in the amygdala. Studies in the hippocampus and barrel cortex have revealed that activation of NMDA receptors induces LTP-like phenomena and causes an insertion of AMPA receptors into synapses (Heynen et al 2000; Shi et al 1999; Takahashi et al 2003). By labeling surface receptors with biotin or with membrane fractionation approaches, Gean et al found that fear conditioning results in an increase in surface expression of the glutamate receptor 1 (GluR1) subunit of AMPA receptors in the lateral and basolateral amygdala (unpublished results). The increase was detected at 2 hours after training and sustained for at least 24 hours. Next, they tested whether fear extinction was accompanied by a concomitant reversal of glutamate receptor protein changes. Rats received 10 pairings of light and footshock and 24 hours later were given three sessions of 10 presentations of light in the absence of shock. Memory retention was assessed 24 hours after light-alone trials. Behavioral assessment revealed that light-alone trials caused a significant reduction in the startle reflex. However, the conditioning-induced increase in surface GluR1 was not reduced significantly by extinction training. This observation is consistent with the classical idea that extinction cannot be accounted for by the erasure of the original memory.

One possible way to reconcile these divergent results might depend on differences in the interval between fear conditioning and extinction training. As noted previously, in the first paper from the Gean group (Lin et al 2003a) showing parallels of quenching with depotentiation, quenching stimulation began early, 10 min after fear conditioning. Whereas extinction training began 24 hours after training (late extinction) in the subsequent experiments, no physiological correlate was examined in those experiments, and the recent unpublished results cited in the previous paragraph suggest that some synaptic changes are not reversed. It is thus possible that extinction beginning within minutes of conditioning (early extinction) and late extinction differ in mechanism. Early extinction might erase the fear association, whereas late extinction might depend on other inhibitory mechanisms that leave the original fear association more or less intact. Such divergent mechanisms have been suggested by two recent papers, one of which shows that early extinction is independent of LVGCCs, whereas late extinction requires them (Cain et al 2005). Another recent paper shows that extinction training that begins within minutes of conditioning shows none of the hallmarks used to argue that the original fear association remains intact: reinstatement, renewal, and spontaneous recovery (Myers et al 2006).

The Expression of Extinction Memory

Consistent with the view of extinction as, at least in part, a reversal of the changes seen with fear conditioning itself, several other molecular changes that follow fear conditioning in basolateral amygdala seem to reverse with extinction training. Specifically, the expression of two molecules involved with GABAergic inhibitory learning are changed by fear conditioning, and those changes are reversed by extinction training. In particular, the mRNA that encodes the $GABA_A$ receptor clustering protein, gephyrin, decreases in BLA after fear conditioning (Ressler et al 2002), as does its protein level and the levels of $GABA_A$ receptors expressed at the cell surface, as reflected by radioactive benzodiazepine binding (Chhatwal et al 2005b). Conversely, gephyrin mRNA and protein levels increase with extinction training, as does the level of benzodiazepine binding in BLA (Chhatwal et al 2005b). Consistent with these molecular results, experiments with BLA infusion of picrotoxin, a $GABA_A$ receptor antagonist, dose-dependently block the expression of extinction, without affecting the expression of non-extinguished fear, suggesting that increases of $GABA_A$-dependent inhibitory neurotransmission are specific to extinction learning (Shekib Jami and MB, unpublished results).

Physiological Correlates of Extinction in the Amygdala

A substantial contribution to our understanding of the role of LA in fear extinction came from two in vivo electrophysiology experiments. In one, short latency (<20 msec after tone onset) cell spiking in LA was increased by fear conditioning and reduced by extinction (Quirk et al 1995). Although increases in spiking during aversive conditioning had previously been seen in recordings from the basolateral (Maren et al 1991) and central nuclei (Pascoe and Kapp 1985), the findings from Quirk et al are particularly important in showing changes specifically in LA, the amygdaloid nucleus with the earliest response to auditory stimuli (Bordi and LeDoux 1992) and the proposed site of convergence of auditory (CS) and somatosensory (US) inputs (Romanski et al 1993). In addition, they show changes in cell spiking that are almost completely reversed by extinction, suggesting that the changes mediating extinction must be upstream of or in LA and suggest that they might be in the same circuit as fear conditioning itself, perhaps even reversing it. In another in vivo electrophysiology study, electrical field responses to the CS increased in LA with fear conditioning and decreased during extinction (Rogan et al 1997). These studies are most often cited for providing clear

evidence for the correlation of cellular responses in LA to fear conditioning. However, they provide equally strong evidence for the correlation of LA activity with fear extinction.

As for infusion studies, the interpretation of in vivo electrophysiological experiments requires considerable care. Although these results convincingly demonstrate that LA cells faithfully report extinction by decreased responding, they cannot localize the source of this change, which could be occurring in LA or could be occurring anywhere upstream of cells there. Quirk et al attempted to forestall this criticism by looking at changes of cell coupling within the LA, measured during spontaneous activity and during CS presentations. Indeed changes in cell coupling were measured in LA units after fear conditioning, including both increased short latency coupling during stimulation and spontaneous synchrony (Quirk et al 1995). Although the short latency coupling disappeared with extinction, the increases in synchrony during spontaneous firing did not. It remains difficult, however, to say whether these changes conclusively indicate a role for LA in extinction learning or memory.

Similarly, the interpretation is problematic for the several human experiments showing amygdala activation during fear extinction. For example, in a protocol in which human subjects were fear conditioned to one stimulus (CS+) by pairing it to an electric shock but not to another (CS−), differential amygdala activation to the CS+ was observed both during acquisition and during early extinction (LaBar et al 1998). These findings have been replicated twice with similar results; the amygdala is activated both during acquisition and early during fear extinction (Knight et al 2004; Phelps et al 2004). These findings strongly indicate that the amygdala plays a role in the extinction learning, although they cannot be called definitive, because amygdala activation early during extinction might simply reflect fear, which is great both during acquisition and during the beginning of extinction training with a CS.

Physiological Models of Extinction Involving the Amygdala

As we have seen, substantial physiological evidence indicates that extinction learning involves and depends on changes in neurotransmission within the basolateral amygdala, perhaps corresponding to alterations of the synaptic excitation of cells there. This idea maps closely onto the common view of a mechanistic relationship between changes in synaptic strength, learning, and behavior. However, as noted in the introduction, substantial behavioral evidence indicates that extinction is inhibitory learning that does not erase the preexisting association of the CS with the aversive US but rather inhibits it in a time- and context-dependent manner (Bouton 1993, 2002), that is, extinction gates the expression of fear.

Such gating might be realized in the brain by several mechanisms. One hypothesis for how such gating might work has followed the description of the role of infralimbic prefrontal cortex in extinction (Milad and Quirk 2002; Quirk et al 2000) and the projection of cells in this area to the intercalated inhibitory interneurons of the amygdala that gate neurotransmission between BLA and central nucleus (Royer and Pare 2002). In fact, prefrontal cortical stimulation reduces the input sensitivity of central nucleus cells (Quirk et al 2003). These researchers also observe that there is no direct projection of LA neurons to the central nucleus and therefore propose that the major contribution of the facilitation of LA cell spiking after fear conditioning is to disinhibit central nucleus firing by changing the efficacy of the intercalated cells.

However, many of the molecular data reviewed here suggest that fear extinction learning occurs within the basolateral amygdala itself. Physiological data suggest how such extinction learning might occur autonomously within the LA. The generation of LTP in LA itself is gated by the suppression of feedforward inhibition from thalamic inputs to the LA principal cells via inhibitory interneurons (Bissiere et al 2003). Furthermore, this feedforward inhibitory pathway demonstrates long-term potentiation itself at both the input to inhibitory cell synapse and at the inhibitory synapse onto the principal cell (Bauer and LeDoux 2004). This suggests the hypothesis that extinction learning might be represented by a long-term potentiation of one or both synapses in the feedforward inhibitory circuit (Figure 1). This inhibition would act in parallel to the long-term potentiation of the excitatory synapse that represents fear learning. By suppressing or eliminating the extinction-induced strengthening of this parallel inhibitory pathway, protocols evoking spontaneous re-

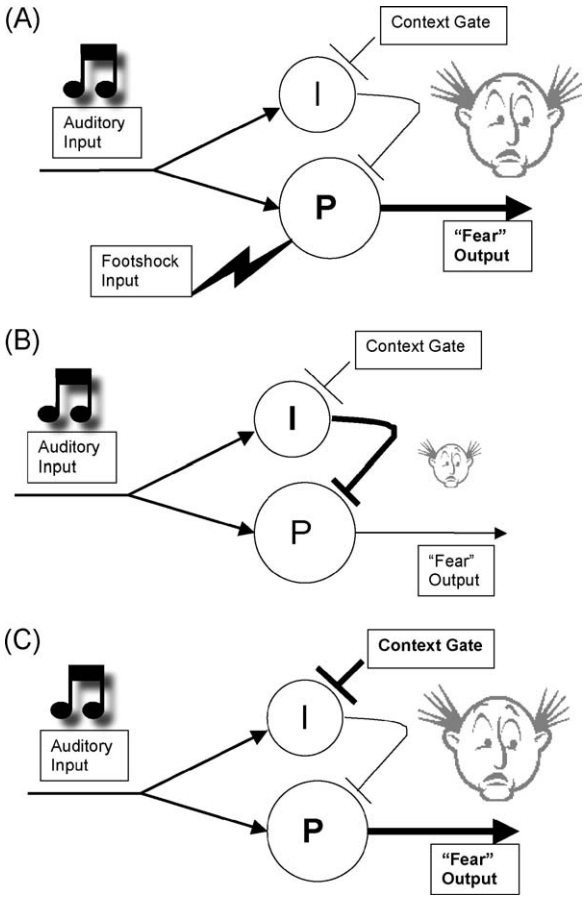

Figure 1. Feedforward model of a local extinction circuit. **(A)** When auditory input is paired with footshock, the synapse onto the principal cell (P) is strengthened, resulting in a strong fear output (bold output axon). **(B)** Multiple presentations of the auditory signal alone during extinction result in strengthening of the inhibitory pathway at the excitatory synapse onto the inhibitory interneuron (I) or at the inhibitory synapse onto the principal neuron. This then inhibits the fear without weakening the associative strength of the excitatory pathway (bold inhibitory axon, light output axon). **(C)** When the "contextual gate" is active (bold inhibitory axon), indicating a change from the time, place, or safety of the extinction context, it inhibits the inhibitory pathway (light inhibitory axon) and allows return of fear in the phenomena in spontaneous recovery, renewal, and reinstatement (bold output axon).

covery, reinstatement, or renewal could uncover the intact excitatory connection and an intact fear response.

Each of the previous hypotheses is concrete and testable through a combination of behavioral and electrophysiological experiments. Such experiments will move the field of extinction studies beyond the question of whether the amygdala makes an important contribution to the learning or memory of extinction to the more sophisticated question of how and what do specific cells of the amygdala contribute to extinction learning.

Summary

Extinction of fear is clearly some form of new learning superimposed on a more or less intact fear association. Fear extinction, like its acquisition, depends on the amygdala. Numerous studies demonstrate that various molecular mechanisms acting within the basolateral amygdala are essential for extinction learning. These mechanisms include NMDA receptors (Falls et al 1992), calcineurin (Lin et al 2003b), MAP kinase (Lin et al 2003c; Lu et al 2001), translation, PI3 kinase (Lin et al 2003c), CB1 receptors (Marsicano et al 2002), LVGCCs, and $GABA_A$ receptors (Cain, Jami, Ponnusamy, and MB, unpublished). In addition to the infusion studies that localize a role for these systems within the amygdala, electrophysiological studies have demonstrated correlated changes in both cell firing and in extracellular potentials that follow the induction and extinction of conditioned fear (Quirk et al 1997; Rogan et al 1997), although other cells increase their CS responses with conditioning but do not revert with extinction (Repa et al 2001). Finally, there are biochemical changes in basolateral amygdala that parallel the behavioral changes of fear extinction, including changes in calcineurin, phospho-AKT, and phospho-CREB (Lin et al 2003b, 2003c), in endocannabinoid levels (Marsicano et al 2002) and in surface expression of $GABA_A$ receptors and of the clustering molecule, gephyrin (Chhatwal et al 2005b). There is thus substantial evidence for a role of the amygdala in extinction learning and in storing the memory of extinction. This role does not exclude contributions by other regions of the brain, including the prefrontal cortex. However, a local circuit to realize extinction within the same structure as acquisition is both efficient and consistent with the expression of extinction in all animals, including in organisms that have neither cortex nor amygdala but only ganglia, such as the snail, Limnaea (Sangha et al 2003, 2004).

This work was supported, in part, by National Institute of Mental Health Grant MH64532 (to MB), National Science Council, Taiwan, Grant NSC94-2752-B-006-001-PAE (to PWG), and German Research Council Grant LU755/1-3 (to BL).

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