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The Amygdala and Fear Conditioning: Has the Nut Been Cracked?

Minireview

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Pavlovian fear conditioning is a ubiquitous form of learning that involves the association of stimuli and their aversive consequences. Perhaps the quintessential example of Pavlovian fear conditioning is Watson and Rayner's (1920) experiment with "Little Albert." In this experiment, Albert, a normal and healthy child attending day care, was shown a white rat by Rayner. Not surprisingly, Albert's first reaction to the white rat was curiosity, and when presented with the rat he reached out to touch it. In response to Albert's attempt to touch the rat, Watson, who had been closely observing Albert's interaction with the rat, sounded a loud and frightening noise by hammering an iron rail. Albert, startled and scared by the noise, quickly withdrew from the rat and began crying. Watson and Rayner continued the procedure, and after a few more presentations of the white rat followed by noise, Albert began to show an intense fear of the rat. Evidently, Albert had associated the frightening noise with the white rat. Indeed, Little Albert had been conditioned to fear white rats!

In recent years, the process by which the brain mediates Pavlovian fear conditioning has come under intense examination. In the laboratory, Pavlovian fear conditioning is typically studied in the object of Albert's fear, the rat. In this model, rats receive pairings of an innocuous conditioned stimulus (CS), such as a tone or the context of the conditioning chamber, and a noxious unconditioned stimulus (US), such as a footshock. After a few such pairings, the CS comes to elicit a constellation of conditioned responses (CRs) that are characteristic of fear, including changes in heart rate and arterial blood pressure, somatomotor immobility (freezing), hypoalgesia, potentiated acoustic startle, and pupillary dilation. In this minireview, we will present recent work that has advanced our understanding of the basic neurobiological mechanisms involved in fear conditioning. This work includes the elucidation of anatomical circuits underlying fear conditioning, the characterization of neuronal and synaptic plasticity in fear conditioning circuits, and the analysis of humans with damage in brain structures required for fear conditioning. Altogether, it has become apparent that neurons in the amygdala, an almond-shaped group of nuclei buried deep within the temporal lobes, are critical for Pavlovian fear conditioning. Hence, it is the amygdala that is likely to have been responsible for Little Albert's fear of rats.

Neuroanatomy of Fear

Although the neural substrates of fear conditioning have received considerable attention in the last decade, they have been under study for over 50 years. Perhaps the first clues to the neural substrates of fear came from the studies of Kluver and Bucy (1937). These investigators found that temporal lobe resections in monkeys produced an eclectic deficit, appropriately termed the Kluver–Bucy syndrome, that was characterized by visual agnosia, hypersexuality, reduced neophobia, and, importantly, loss of fear. Later work indicated that the reduced fear in resected monkeys was due specifically to damage in the amygdala. Consistent with its general role in fear, reports began to emerge that the amygdala was also required for aversive learning, including the acquisition of conditioned avoidance responses in cats and conditioned emotional responses in rats. Together, these reports provided a strong foundation for amygdaloid involvement in fear and aversively motivated learning.

Building upon this foundation, considerable progress has been made in the last decade further defining the anatomy of the amygdaloid fear system (Figure 1). It is now apparent that within the amygdala there are two subsystems that have unique roles with regard to fear conditioning (Davis et al., 1994; Fanselow, 1994; LeDoux, 1995). The basolateral complex of the amygdala (BLA; comprised of the lateral [LA], basolateral [BL], and basomedial [BM] nuclei) is a substrate for sensory convergence from both cortical and subcortical areas, and is considered a putative locus for CS–US association during fear conditioning. In contrast, the central nucleus of the amygdala (CE), which receives projections from the BLA, projects to brain areas involved in the generation of fear responses, such as the lateral hypothalamus (LH) and periaqueductal gray (PAG). It is therefore thought to be a final common output pathway for the generation of fear CRs. Consistent with these roles, destruction of neurons in either the BLA or CE is detrimental to both the acquisition and expression of conditional fear (Campeau and Davis, 1995; Maren et al., 1996), regardless of the exact stimuli used to train fear or the response measure used to assess it. Thus, the amygdala is ideally situated to both integrate and associate sensory information and to execute motor programs during fear conditioning.

Associative Neuronal Firing in the Amygdala

It has been known for years that amygdaloid neurons respond to conditional reinforcers. However, the recent delineation of the amygdaloid circuits underlying fear conditioning has opened the door for fine-grained studies of physiological plasticity in these circuits during learning. For example, investigations of neuronal firing in the amygdala during aversive learning using multiple-unit recording techniques have revealed learning-induced changes in both the CE (Applegate et al., 1982) and BL (Maren et al., 1991). In both cases, neuronal discharges were significantly greater to an auditory CS that was paired with a shock US than those to a different CS that was not paired with shock.

Although neuronal discrimination suggests that the changes in neuronal firing in the amygdala were associative in nature, it is not clear whether the associative activity was generated at the recording site or relayed from an afferent region. Indeed, within the amygdala, the first locus of convergence for auditory CSs and footshock USs is in the LA. In recent experiments,

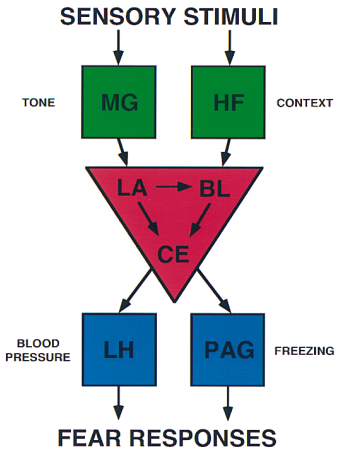

Figure 1. The Amygdaloid Fear System

Unimodal and polymodal sensory stimuli enter the amygdala (red) from both subcortical and cortical relays (green). For tone and context stimuli, sensory information is conveyed by the medial geniculate nucleus of the thalamus (MG) and the hippocampal formation (HF), respectively. Projections from these sensory relays target the lateral (LA) and basolateral (BL) amygdaloid nuclei, which, in turn, project to the central nucleus of the amygdala (CE). The CE projects to brain structures (blue) involved in the generation of fear responses. For example, the lateral hypothalamus (LH) and periaqueductal gray (PAG) mediate increases in blood pressure and freezing, respectively.

LeDoux and his colleagues (Quirk et al., 1995) have used parallel single-unit recording techniques to analyze neuronal firing in the LA during Pavlovian fear conditioning in rats. Compared with rats receiving unpaired tone CSs and footshock USs (a nonassociative control), rats receiving tone–shock pairings exhibited significant increases in single-unit activity in the LA. Interestingly, this increase in unit firing was expressed at relatively short latencies (<15 ms) from tone onset, which contrasts with the much longer latency of conditional multiple-unit activity in the CE and BL. In addition, fear conditioning induced changes in both the coupling of cell pairs and the interspike intervals of single cells (which persisted despite extinction training) in the LA, suggesting its involvement in associative memory formation. Together, these pieces of evidence suggest that the LA may be the initial site for training-elicited plasticity during fear conditioning with tone CSs. Of course, it is also possible that associative plasticity occurs in brain structures afferent to the amygdala, such as the medial geniculate nucleus of the thalamus (MG; Weinberger, 1995).

Synaptic Plasticity in the Amygdala

What is the basis for associative changes in amygdaloid unit activity during fear conditioning? One possibility is that fear conditioning results in an activity-dependent enhancement of synaptic transmission (e.g., long-term potentiation or LTP) at synapses formed on amygdala neurons by axons carrying CS information. By this model (Figure 2), coincidental activity in an initially "weak" CS pathway and a "strong" US pathway would yield LTP at CS–BLA synapses, consequently enabling CR production. As a first step towards verifying this model, it has now been demonstrated that LTP can be induced in the

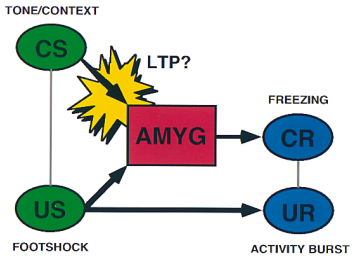

Figure 2. Proposed Role for LTP in CS–US Association Formation in the Amygdala

The amygdala (red) is a substrate for the convergence of sensory information concerning conditional (CSs; tones and contexts) and unconditional (USs; footshocks) stimuli (green). Concurrent activity in CS and US pathways to the amygdala is hypothesized to permit the induction of long-term potentiation (LTP) in the CS pathway. Enhancement of synaptic transmission in CS–AMYG projections allows the CS to elicit conditional responses (CRs), such as freezing, via projections of the amygdala to response-generating structures (blue). Unconditional responses (URs) to the US, such as footshock-elicited activity bursts, do not require the amygdala for their expression and are elicited by direct projections from sensory structures to response structures.

BLA in vivo by high frequency stimulation of excitatory afferents from putative CS pathways, including the MG (Clugnet and LeDoux, 1990) and hippocampal formation (HF; Maren and Fanselow, 1995). Moreover, Rogan and LeDoux (1995) have recently demonstrated that LTP induction in the MG–LA projection increases the amplitude of auditory evoked potentials, which arrive in the amygdala via the MG. This indicates that experimentally induced increases in synaptic efficacy can affect the processing of physiological stimuli that use the potentiated synapses. However, experiments have yet to be performed to determine whether coincidental activity in putative CS and US pathways is sufficient for generating "associative" LTP in the BLA.

Additional support for a role of BLA LTP in fear conditioning is provided by experiments demonstrating that infusion of D,L-2-amino-5-phosphonovalerate (APV), an N-methyl-D-aspartate (NMDA) receptor antagonist, into the BLA prevents both LTP induction in the BLA (Maren and Fanselow, 1995) and fear conditioning to contextual (Fanselow and Kim, 1994) and discrete (Miserendino et al., 1990) CSs. Intra-amygdala APV infusion apparently does not affect the performance of conditional fear in trained subjects (Miserendino et al., 1990), consistent with the role of NMDA receptors (at least those in the hippocampus) in the induction, but not expression, of LTP. However, recent physiological work indicates that NMDA receptors in the BLA may be involved in regulating cell excitability in vivo (Li et al., 1995; Maren and Fanselow, 1995), leaving the effects of intra-amygdala NMDA receptor antagonists on the acquisition and expression of fear conditioning an open question. Nonetheless, the available evidence supports a role for amygdaloid LTP in the acquisition of Pavlovian fear conditioning.

The Amygdala and Fear Conditioning in Humans

As we have seen, animal models of Pavlovian fear conditioning strongly implicate the amygdala in this form of learning and memory. To ascertain the role of the human

amygdala in fear conditioning, a recent study from Damasio and his colleagues (Bechara et al., 1995) used a design very similar to that of the Little Albert experiments, but combined it with knowledge of the amygdala's involvement of fear gleaned from the animal work. In this study, a patient (S. M.) with Urbach–Wiethe disease, a rare disorder that results in bilateral degeneration of the amygdalae, was subjected to fear conditioning using either visual or auditory CSs and a loud horn as a US; skin conductance served as the measure of conditional fear. Compared with normal control patients, S. M. showed no evidence of fear conditioning to either the auditory or visual CS. Nevertheless, S. M. showed normal unconditioned responses (URs) to the horn US, suggesting that her deficit in conditioning was not a performance problem.

Despite her fear conditioning deficit, S. M.'s recall of events associated with fear conditioning was intact; that is, she could accurately describe both the training procedures and the causal relationship between the conditional and unconditional stimuli. In contrast to S. M., a patient with selective hippocampal damage showed normal fear conditioning but impaired recall, whereas another patient with combined amygdala and hippocampal damage showed neither normal fear conditioning nor intact recall. Collectively, these findings suggest that there are anatomically distinct neural systems mediating different aspects of the task, with the amygdaloid system playing a critical role in the acquisition of Pavlovian fear conditioning and the hippocampal system mediating declarative memory for the events associated with training. A role for the human amygdala in fear has also been suggested in recent studies in which patients with Urbach–Wiethe disease were impaired in recognizing fear in facial expressions (Adolphs et al., 1995). Fear conditioning in humans is also impaired by unilateral temporal lobectomies, which produce substantial amygdala damage (LaBar et al., 1995).

Has the Nut Been Cracked?

The foregoing discussion reveals that our understanding of the basic neurobiological mechanisms of aversive learning has advanced considerably. However, there are still a number of issues that remain to be tackled. First, is the amygdala a storage site for conditional fear memories? In favor of this hypothesis, recent data indicate that the amygdala has a long-term role in expressing fear conditioning over time. We have recently found that selective neurotoxic lesions of the BLA, which spared the CE (a critical point given the role of the CE in fear performance), produce deficits in the expression of conditional fear when made up to 28 days after training (Maren et al., 1996). Kim and Davis (1993) have reported a similar result with electrolytic CE lesions. Hence, these reports are consistent with the storage of aversive memories in the amygdala. In contrast, McGaugh and his colleagues have evidence that the amygdala has a temporary role in the consolidation of aversive memories (McGaugh, 1989). Therefore, additional work is required to determine under what conditions the amygdala has an enduring versus a temporary role in fear conditioning.

Second, is the amygdala involved in learning, performance, or both? That is, the deficits in both the acquisition and long-term expression of fear conditioning produced by either CE or BLA lesions do not reveal a specific role for these amygdaloid nuclei in either the learning or performance of conditional fear. However, the identification of CS–US convergence in the LA (Romanski et al., 1993), the rapid development of short-latency associative neuronal firing in the LA (Quirk et al., 1995), the selective of effects of intra-amygdala NMDA receptor antagonists on the acquisition of conditional fear (Miserendino et al., 1990), the discovery of NMDA receptor-dependent LTP in the BLA in vivo (Maren and Fanselow, 1995), and the selective effects of damage to the human amygdala on fear CRs (Bechara et al., 1995) favor the amygdala as a learning structure. Nonetheless, these findings do not preclude a role of the amygdala in the performance of fear responses, and the effects of amygdala lesions on innate or unconditioned fear (e.g., Blanchard and Blanchard, 1972; Adolphs et al., 1995) seem consistent with this possibility. Perhaps the amygdala is required for both the learning and performance of conditional fear, functions that may be mediated by the BLA and CE, respectively.

Third, does LTP in the BLA underlie the acquisition of Pavlovian fear conditioning and associative neuronal discharges in the amygdala? As discussed in this minireview, there are now a number of provocative findings that suggest a role for LTP in the amygdala in the acquisition of fear conditioning; it is tempting to speculate that LTP is also responsible for the development of conditional neuronal activity in the amygdala during learning. However, there are lessons to be learned from a close examination of other attempts to link synaptic plasticity mechanisms with learning, for example, efforts to link hippocampal LTP and spatial learning. As Barnes (1995) has pointed out, what has frequently been taken as strong evidence for a role of hippocampal LTP in spatial learning has later been shown to have limited validity or has been explained as a spurious correlation. In retrospect, this work has demonstrated that the task of linking synaptic plasticity with learning is exceptionally difficult. Thus, caution must be exercised when making claims that LTP in the amygdala underlies fear conditioning. Indeed, considerably more work will be required to understand the extent to which LTP in the amygdala serves as a mechanism for Pavlovian fear conditioning.

And, fourth, how do neuronal ensembles in the amygdala encode CSs, and how do these codes translate into learned behavior? Now that we have begun to identify neuronal correlates of aversive learning in the amygdala, important questions for future research are the nature of the ensemble firing patterns that encode CSs in the amygdala and the translation of these ensemble codes into the diversity of fear CRs observed following training. Single-unit recordings in LA have begun to reveal how pairs of neurons in the amygdala might encode CSs (Quirk et al., 1995), but the translation of neuronal firing in the amygdala into behavioral CRs is a problem that has yet to be addressed. Clearly, further detailed physiological investigations of neuronal activity in the amygdala and interconnected structures are required to begin to answer these important questions.

Despite these unresolved issues, however, there is general consensus that the neurons in the amygdala are necessary for the acquisition of Pavlovian fear conditioning. The recent and exciting findings discussed in this minireview bring us one step closer to understanding the basic neurobiological processes underlying this

important form of behavioral plasticity. And although we have made considerable progress in understanding these mechanisms, there is still much to be done before we crack the brain's almond.

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