

# Why We Think Plasticity Underlying Pavlovian Fear Conditioning Occurs in the Basolateral Amygdala

## Viewpoint

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Activation of the brain's fear system transforms an animal into an effective defender against environmental threat (e.g., Bolles, 1970; Bolles and Fanselow, 1980; Fanselow, 1994; LeDoux, 1996). The fear system has been most systematically explored using Pavlovian fear conditioning, a procedure in which emotionally neutral stimuli that occur in connection with harmful or otherwise aversive events acquire the capacity to elicit defensive responses. In laboratory studies, the aversive event, typically footshock, is called the unconditional stimulus (US) and the neutral stimulus the conditional stimulus (CS).

Many investigators, including the authors of this paper, have argued that neural plasticity occurring within the basolateral complex of the amygdala (ABL) encodes the emotional component of the memories formed during fear conditioning (LeDoux, 1996; Maren and Fanselow, 1996; Davis, 1997). It has also been suggested that the ABL modulates the consolidation of memories formed in other brain regions (Cahill and McGaugh, 1998). There is nothing inherently exclusive about these two positions. The ABL may acquire and store information about the aversive experiences that activate it, and one effect of this may be the enhancement of memory consolidation in other brain areas that represent and store different (nonemotional) aspects of the experience. However, one version of the modulatory view argues that the amygdala only modulates memories stored in other brain regions and has no direct role in acquiring or storing memories (Cahill and McGaugh, 1998; Vazdarjanova and McGaugh, 1998). In the following discussion, we argue that the available evidence suggests the amygdala learns and stores information about fear-arousing events but also modulates storage of other types of information in different brain regions.

### Evidence for Encoding

In order for the ABL to be identified as a site of memory encoding and storage, several conditions would have to be satisfied. First, destruction of the ABL before training should prevent learning, whereas lesions made after training should prevent retention of fear memory. Second, neural activity in the ABL should be modified by experience and specifically should change during the learning process as the memory is established. This altered activity should manifest itself during testing of fear. Third, learning should not occur if neural activity

in the ABL is temporarily disrupted only during learning. Each of these conditions has been satisfied.

### *Destruction of the ABL before Training Prevents Learning*

Lesions of the amygdala produce a pronounced and often complete loss of a large number of Pavlovian conditional fear responses, including freezing, potentiated startle, analgesia, hypertension, and ultrasonic vocalizations (Kapp et al., 1979; LeDoux et al., 1988; Davis, 1992; Helmstetter, 1992; Fanselow, 1994; Goldstein et al., 1996). Lesions restricted to the ABL even a month after training eliminate conditional fear responses, which is consistent with the idea that the ABL plays a role in the long-term storage of information relevant to fear conditioning (Lee et al., 1996; Maren et al., 1996b). Since lesions made both a week before training and a month after training have similarly devastating effects, the results cannot be explained by reference to consolidation processes that occur immediately after training (Maren et al., 1996b).

When assessing the effects of brain damage on conditional fear or any other learning and memory task, it is important to ask whether the lesion interferes with the associative or nonassociative aspects of the task. The former are attributable to the learned relation between the CS and the US, whereas the latter are simply due to the sensitizing or arousing effects of the US. The use of proper control procedures allows these factors to be separated (e.g., Rescorla, 1967). For example, lesions of the lateral amygdala (part of the ABL) reduce freezing and blood pressure responses elicited by the CS to the same level as that seen in a nonassociative control group trained with unpaired CS-US presentations (LeDoux et al., 1990). This suggests that such lesions do not interfere with the ability to perceive the CS and US or to express fear responses, but instead interfere with the ability to form an association between the CS and the US.

Vazdarjanova and McGaugh (1998) recently reported that ABL lesions virtually eliminated freezing but only partially disrupted the rats' preference for one compartment of a 3-arm maze where shock did not occur. While Vazdarjanova and McGaugh interpret the residual avoidance of the shocked arm in ABL-lesioned animals as evidence of undisrupted fear conditioning, there is an important caveat to the interpretation of this data. Since their experimental design does not produce conditioning-specific avoidance of the shocked arm in the sham-operated control animals, one cannot assess associatively based fear responses in the ABL-lesioned rats.

While many of the lesion studies in the literature that have implicated the ABL in fear conditioning have used freezing behavior as the measure, other indices of fear have been used as well. These include potentiated startle, heart rate, hypertension, analgesia, ultrasonic vocalization, and defecation (Kapp et al., 1979; LeDoux et al., 1988; Davis, 1992; Helmstetter, 1992; Fanselow, 1994; Goldstein et al., 1996). Damage restricted to the ABL has been shown to prevent conditioning for several of

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these responses, including hypertension and potentiated startle as well as freezing. For many of these measures, unconditional responses (responses elicited by the US) are unaffected by lesions of the ABL or other amygdala areas, demonstrating that the effects of amygdala damage on conditional and unconditional responses can be dissociated. This indicates that the ABL is not required for the expression of responses in these modalities per se but instead for the engagement of these modalities by conditional fear stimuli. This conclusion is also supported by the studies described above showing that, following ABL lesions, nonassociative freezing and blood pressure responses elicited by the CS are intact (LeDoux et al., 1990).

Nevertheless, for Pavlovian conditional fear responses elicited by a conditional fear stimulus, it is true that the ABL is required to mediate between the sensory world and motor effectors. As a result, lesion studies alone cannot provide complete justification for the view that the ABL is involved in learning, since the same lesion that prevents the acquisition of the association will also prevent the behavioral expression of that learning. However, when the lesion results are combined with other convergent lines of evidence, the case is substantially strengthened.

#### ***Neural Activity Changes in ABL during Learning as the Memory Is Established***

Fear conditioning induces changes in the electrophysiological responses of cells in the ABL. Thus, the response of cells in the ABL to a tone CS, or to electrical stimulation of pathways that transmit CSs to the ABL, is enhanced following Pavlovian fear conditioning (e.g., Quirk et al., 1995, 1997; McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997). Further, plasticity, in the form of long-term potentiation (LTP), occurs in the CS pathways to the ABL (Clugnet and LeDoux, 1990; Maren and Fanselow, 1995; Huang and Kandel, 1998; M. G. Weisskopf and J. E. LeDoux, 1998, Soc. Neurosci., abstract). Particularly significant is the fact that fear conditioning and LTP induction produce very similar changes in neural activity and that these track the emergence of behavioral fear responses (Rogan et al., 1997).

Neural activity has also been shown to change during fear conditioning in sensory structures afferent to the amygdala, such as the auditory cortex and auditory thalamus (e.g., Weinberger, 1995; Quirk et al., 1997; Armony et al., 1998), raising the possibility that the essential plasticity occurs in these areas. However, plastic changes develop in fewer trials in the ABL than in the auditory cortex (Quirk et al., 1997), and lesions of the amygdala that include the ABL eliminate aspects of cortical plasticity (Quirk et al., 1997; Armony et al., 1998). Although studies have not been performed comparing plasticity in the auditory thalamus with the amygdala, findings to date from the cortical studies suggest that plasticity in sensory areas depends on the amygdala rather than the other way around.

Since LTP in the ABL interacts with paired-pulse facilitation in vivo, Maren and Fanselow (1995) suggested that this form of LTP was expressed presynaptically. More recent studies using in vitro brain slices are consistent with the idea that presynaptic terminals of thalamic and cortical afferents in the amygdala participate in synaptic plasticity (McKernan and Shinnick-Gallagher, 1997;

Huang and Kandel, 1998). However, the involvement of presynaptic neurons does not mean that the essential plasticity occurred in the thalamus or cortex rather than in the ABL. Presynaptic plasticity involves synapses that are located in the terminal region. Further, to the extent that the physiological changes that occur in the ABL during conditioning are associative in nature (Quirk et al., 1995; Rogan et al., 1997), and to the extent that associative plasticity involves interactions between pre- and postsynaptic neurons (Hebb, 1949; Brown et al., 1988), then postsynaptic cells in the amygdala must be involved in the learning, even if there is a presynaptic component to the plasticity. Indeed, although Huang and Kandel's (1998) data suggested that LTP in ABL slices was expressed presynaptically, they demonstrated that induction of this plasticity depended on postsynaptic activation.

In some studies, neural activity was found to increase during early training but then "reset" as training continued (Quirk et al., 1997). The firing rate thus goes up initially and then goes back down. While this might be viewed as evidence for a time-limited role of the ABL (Cahill and McGaugh, 1998), three additional points need to be considered. First, while the average of all cells shows a tendency for the ABL to reset, cells that do and that do not reset contribute to this average. Thus, some cells in the ABL continue to express the learned change throughout acquisition. Second, many of the cells that reset during late training exhibited evidence of having been modified when learning was tested after training was complete. That is, the cells responded at the higher, conditional rate when the shock was terminated and performance tested to the CS alone. Similarly, in studies of functional activation in the human brain during fear conditioning, amygdala activity increases in early training, resets as training continues, and then is expressed at the higher conditional level when testing in the absence of the US starts (Buchel et al., 1998; LaBar et al., 1998). The amygdala's role is not temporary. Third, functional connections develop between ABL cells during training, allowing the memory to be encoded by the timing between spikes in addition to being encoded by increases in spiking (Quirk et al., 1995). Temporal coding may constitute an important memory mechanism in the ABL, one that is overlooked by measures of firing rate.

#### ***Disruption of Neural Activity during Learning Prevents Learning***

The neural recordings add considerably to the encoding view, suggesting that ABL is plastic during training. While such findings are correlational rather than causal, additional findings show that neural activity in ABL during acquisition is required for fear conditioning. This evidence comes from studies in which the ABL is pharmacologically manipulated temporarily during training. For example, Gewirtz and Davis (1997) found that acquisition of second-order fear conditioning was blocked by pretraining infusion into the ABL of AP5, a drug that blocks NMDA receptors. Test performance to the first-order stimulus, which was trained in the absence of the drug, was actually enhanced. Further, Muller et al. (1997) infused the GABA agonist muscimol into the ABL immediately prior to training. This drug increases inhibition and thereby functionally inactivates the infused area.

When tested drug free, the rats showed no evidence of having been conditioned.

When drugs are infused into the amygdala before training, the drug will affect processes that occur both during and after training. This is important, since it is well established that posttraining infusion of drugs into the ABL can affect the memory for tasks that depend on the hippocampus or striatum (e.g., Packard et al., 1994). However, Maren et al. (1996a) found that while immediate pretraining infusion of AP5 into the ABL blocked acquisition of conditional fear, immediate post-training infusion of the same drug had no effect. Similarly, A. E. Wilensky et al. (1998, Soc. Neurosci., abstract) injected muscimol into the ABL immediately before or immediately after training and found that the subsequent expression of learned fear was only affected by pretraining infusions. Since these and other studies (e.g., Lee and Kim, 1998) tested rats in a drug-free state (i.e., the amygdala was functional at the time of the test), the amygdala manipulation cannot be attributed to a blockade of expression of freezing. Thus, these drugs have to be having their effects on acquisition as opposed to consolidation or performance.

It is important to point out that the interpretation of the AP5 studies does not hinge on the issue of whether NMDA receptors are involved in transmission or plasticity. Several studies have found that AP5 interferes with synaptic transmission in the ABL (see Maren and Fanselow, 1995; Li et al., 1995), which may explain why infusion of AP5 in the amygdala right before testing can interfere with the expression of fear (Maren et al., 1996a; Lee and Kim, 1998). The only issue that is relevant here is the fact that AP5 infused before training blocks learning, whereas the same treatment after training has no effect.

In sum, the effects of pretraining infusions of muscimol and AP5 into the ABL cannot be attributed to effects occurring after training. Since muscimol injected into the ABL immediately after training attenuates retention of forms of learning that do not depend on the amygdala, the ABL may indeed participate in the modulation of memories encoded by other structures. However, since the same manipulation does not affect fear conditioning, the ABL does not seem to modulate this form of learning. Since we believe that the ABL is involved in the plasticity underlying fear learning, we conclude that the ABL does not modulate itself. However, it may well be modulated by other systems.

#### A Synthesis

The encoding view provides a parsimonious account of the multiple convergent lines of evidence for the ABL's role in fear conditioning. We do not dispute the notion that activation of the ABL can facilitate consolidation of information in other structures, such as the hippocampus, other cortical areas, or the striatum (Cahill and McGaugh, 1998; Packard and Teather, 1998). Rather, we argue that the ABL is involved in the encoding of fear memory and the modulation of memory functions of other structures.

While we believe that the ABL is essential to the implicit learning that constitutes fear conditioning, we are not proposing that all of the plasticity relevant to fear conditioning necessarily occurs within the ABL. It seems

possible that the ABL, while essential, is also part of a distributed network that encodes the fear memory. For example, as noted, there is compelling evidence that plastic changes occur in regions that are afferent to the ABL, such as thalamic and cortical sensory systems that process CSs (see Weinberger, 1995). Additionally, cortical areas that are both afferent and efferent to the ABL (e.g., perirhinal cortex, the hippocampal formation, and sensory cortex) may participate with the ABL in the long-term encoding of fear. It remains for future research to determine whether these distributed representations exist and, if so, to unravel their nature.

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