# Genetics of Childhood Disorders: L. Learning and Memory, Part 3: Fear Conditioning

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Described initially at the beginning of the 20th century by Ivan Pavlov, classical conditioning involves the pairing of neutral stimuli with aversive or appetitive cues. This results in learning. Formally neutral stimuli now predict salient events. One form of Pavlovian conditioning is fear conditioning, the primary associative learning mechanism involved in aversive emotional learning. It is through this process that we learn to be fearful of people, animals, objects, and places. From a psychological perspective, it is one of best understood types of learning in mammals because the stimulus and response properties can be very carefully controlled. For these reasons, Pavlovian fear conditioning has served as a powerful animal model of fear and anxiety disorders including phobia, panic disorder, posttraumatic stress disorder, and possibly many of the "neurotic"

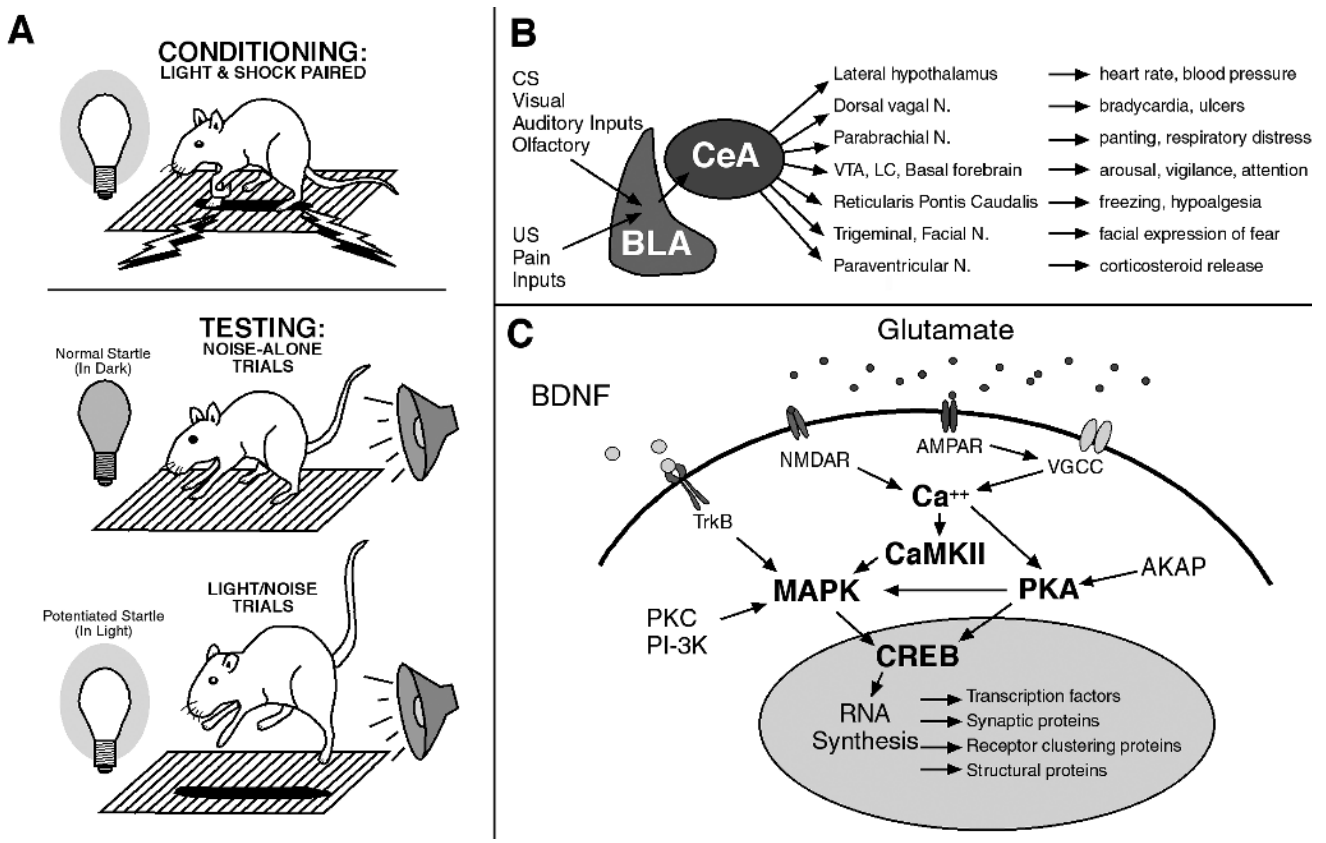


**Fig. 1** (A) Pavlovian fear conditioning and measurement of fear-potentiated startle. Top: during training, the CS (light) is paired with the US (foot-shock). During testing, the acoustic startle reflex is elicited in the absence (middle) or presence (lower) of the CS. Fear-potentiated startle is defined by higher acoustic startle amplitude in the presence of the fear-conditioned CS. (B) Schematic diagram illustrating the convergent inputs of US (pain) pathways and CS (multimodal sensory) pathways into the basolateral nucleus of the amygdala (BLA). Neurons in the basolateral nucleus project to the central nucleus (CeA). Neurons in the central nucleus project divergently to numerous brain regions which together mediate the fear response. CS = conditioned stimulus; US = unconditioned stimulus; VTA = ventral tegmental area; LC = locus ceruleus. (C) Schematic diagram illustrating some of the molecules implicated in the formation of fear memory. In brief, *N*-methyl-D-aspartate (NMDA) and other receptor activation leads to downstream intracellular events, eventually culminating in the transcription of numerous new genes involved in long-term memory consolidation. NMDAR = the *N*-methyl-D-aspartate glutamate receptor; AMPAR = the glutamate receptor which mediates cell depolarization; VGCC = voltage-gated calcium channel; TrkB = the tyrosine kinase receptor for brain-derived neurotrophic factor (BDNF); CaMKII = calcium calmodulin-dependent protein kinase II; MAPK = mitogen activated protein kinase; AKAP = A-kinase anchoring protein; PKA = cyclic AMP-dependent protein kinase; PKC = protein kinase C; PI-3K = phosphatidylinositol-dependent kinase; CREB = cyclic AMP response element binding protein.

associations of everyday life. If we can understand the underlying molecular basis for how a previously innocuous stimulus leads to intense fear in an animal model, the hope is that such an understanding will eventually lead to better treatments in humans disabled by these crippling disorders.

Besides the obvious usefulness of this model system for understanding fear and anxiety disorders, fear conditioning has received considerably more attention in recent years for two reasons. Investigators are able to carefully control the stimulus-response properties of fear conditioning in rodents. This has led to very precise behavioral paradigms for studying learning and memory. In addition, the ability to combine behavioral, anatomical, and electrophysiological techniques, both in vitro and in vivo, has contributed to an advanced understanding of the neural circuitry underlying this form of learning. This combination of approaches has made fear conditioning and its neural substrates among the best models for the understanding of learning and memory across the cellular, neural systems, and organismal levels of organization. In this brief review, we will first describe the experimental paradigm involved in generating and measuring fear-conditioned learning. We will then discuss the neural circuits and their cellular substrates that are thought to mediate the learning and memory process.

In general, fear conditioning involves the pairing of a previously neutral cue, which then becomes the conditioned stimulus (CS), with an aversive cue, the unconditioned stimulus (US). In rodents, the US is typically given in the form of a mild foot-shock (Fig. 1A). Although any form of sensory cue may be used, the CS is typically a discrete tone, light, or odor. In fact, rodents can easily discriminate among numerous discrete cues in this form of conditioning. After several pairings of the CS and US, the animal will display a conditioned response to the CS alone which is indicative of a central state of fear. Any time after this training, from hours to months, a fear response can be elicited by exposure to the CS alone.

The most commonly used measures of conditioned fear are the freezing response and the fear-potentiated acoustic startle reflex. With both of these measures, the animal is placed in a neutral environment and the CS is presented in the absence of the US. Freezing is measured as immobility during the presentation of the CS. The percentage of time spent freezing is found to be significantly greater in the presence than in the absence of a fear-conditioned CS. Alternatively, the acoustic startle reflex is elicited by presenting the animal with a short noise burst which serves to elicit a reproducible startle response (Fig. 1A). Measurement of the startle reflex can be entirely automated such that a computer may be used to record the magnitude of the startle response as well as to control the presentations of the CS and US. In the presence of the CS (e.g., light, odor), the magnitude of the startle elicited by the noise burst is reproducibly greater than it is in the absence of the CS, and this difference is referred to as *fear-potentiated startle.*

The amygdala is the primary brain region involved in fear-conditioned learning. However, "the amygdala" in fact refers to a number of functionally separate nuclei that are anatomically grouped together. The learned state of fear involves activation of the basolateral nucleus of the amygdala which in turn activates the central nucleus of the amygdala (Fig. 1B). As its name implies, the central nucleus is a "hub" that serves to initiate the full fear response whether it is activated physiologically via the basolateral nucleus or experimentally through electrical stimulation.

The "fear response" is generated through the hardwired neural connections that exist between the central nucleus and a number of other neural pathways. For example, activation of various midbrain nuclei by the central amygdala results in freezing, potentiation of reflexes such as the acoustic startle reflex, and increased respiration. Projections to the lateral hypothalamus activate the sympathetic nervous system leading to cardiovascular effects, pupil dilation, and increased sweating. Activation of the paraventricular nucleus of the hypothalamus activates the glucocorticoid response. Lesions of these individual brain regions that are downstream of the central nucleus serve to block specific aspects of the fear response, whereas ablation of the central nucleus itself blocks the entire fear response.

In addition to the well-understood circuitry underlying the outputs of fear response, the sensory inputs representing the CS and US pathways have also been studied. Auditory, visual, and somatosensory projections arrive at the basolateral amygdala (BLA) through both thalamic and cortical pathways. Olfactory pathways, on the other hand, take a more direct route from the olfactory bulb through the piriform cortex and directly into the amygdala. In all cases, the pathways representing the aversive US and the previously neutral CS converge in the BLA. It is within the BLA that the most critical cellular processes underlying associative learning are thought to occur.

There is a large amount of data supporting a role for the BLA in initiating associative fear learning. Chemical and electrical lesions of the BLA have been shown to block new fear learning. Furthermore, the principal electrophysiological measure of learning, long-term potentiation (LTP), appears to occur between the neurons projecting to and the target neurons within the BLA in brain slices. Electrical stimulation of neurons within the BLA before and after fear conditioning in freely moving rats has shown that there is potentiation of the pathway mediating many of the sensory connections to neurons within the BLA. Taken together, these data suggest that fear conditioning may result in an alteration in the functional connectivity between sensory pathways and the BLA, such that future presentation of the CS alone is now sufficient to activate the central nucleus.

One model for the circuitry changes underlying fear conditioning proposes that when the associative CS-US pairing occurs, strong firing of neuronal projections that mediate the aversive stimuli are paired with weaker firing representing the neutral stimuli. This coincident firing leads to a number of

events that result in the strengthening of the BLA connections that represent the neutral stimuli. The strengthening of BLA connections could occur through at least two mechanisms: either increasing the efficiency of presynaptic neurotransmitter release, or increasing the sensitivity of postsynaptic terminals through structural modifications at the synapse. An active area of research is aimed at determining whether the alteration in neuronal connectivity is limited to changes in synaptic efficacy, to actual structural change, or to both mechanisms.

The cellular events that mediate the associative learning between axons representing the CS and US pathways are also beginning to be understood (Fig. 1C). The glutamatergic *N*-methyl-D-aspartate (NMDA) receptors are thought to perform the primary function of mediating associative learning at the level of the synapse. Blockade of these receptors has been shown to block the acquisition of new fear associations, but not to block the expression of previously learned fear responses. As with other memories, fear conditioning appears to have short-term and long-term phases of consolidation. Short-term memory formation appears to be dependent on a number of receptors including NMDA receptors, voltage-gated calcium channels, and norepinephrine receptors. In contrast, the transition from short-term to long-term representation appears to require the addition of new mRNAs and protein synthesis.

The initial cellular events that occur following the coincident association of the CS and US involve an influx of calcium into the postsynaptic neuron. This occurs when the NMDA receptor is activated through glutamatergic release as well as when the voltage-gated calcium channel is activated by membrane depolarization. In addition, other neurotransmitters such as norepinephrine and growth factors such as brain-derived neurotrophic factor (BDNF) have been implicated in the activation of the neuronal events mediating memory formation. A number of intracellular factors are induced by receptor activation during CS-US pairing including kinases, phosphatases, and transcription factors. Some of the better understood pathways are illustrated in Figure 1C.

A central point is that the signaling cascades that are activated lead ultimately to the phosphorylation of transcription factors within the nucleus. It is thought that these early events initiate long-term memory formation by facilitating a later wave of mRNA and protein synthesis, involving the production of other transcription factors, synaptic clustering molecules, and molecules that mediate axonal or dendritic structural plasticity. Through this cascade of events—occurring over time courses ranging from milliseconds to days—the associative pairing of the CS and US leads to alterations in synaptic efficacy. This results in a stronger activation of the basolateral and central amygdala following presentation of a seemingly neutral stimulus (the CS) than occurs before conditioning.

We have outlined the role of the BLA as a site for convergence of CS and US representations and as a likely site for mediating the cellular and molecular processes underlying fear learning. Much progress has been made as numerous investigators have focused on this brain area using fear conditioning as a behavioral tool. Undoubtedly, this approach will continue to provide an excellent model of learning as the amygdala appears to serve as an integrator between input associations and output behavior.

The amygdala is not the only region in the brain undergoing changes when fear conditioning takes place. Accumulating evidence suggests that salient learning alters other brain areas such as limbic, cortical, and subcortical regions. Furthermore, there is a large body of literature devoted to the role of the amygdala in activating the norepinephrine system when a fearful event occurs, and it is clear that this and other neurotransmitters participate in consolidating memories in multiple brain regions.

In summary, Pavlovian fear conditioning is a simple model of associative learning. It serves as a powerful experimental tool because of its position at the interface between behavior and neurobiology. Behaviorally, precise experimental control is possible through subtle manipulation of sensory cues. Neurobiologically, the amygdala is an important mediator of convergent sensory associations and a source of divergent outputs related to learned fear. Numerous investigators have used this circuitry to show that fear conditioning may be dependent on LTP and is mediated by both short-term and long-term consolidation processes. Finally, rapid progress in understanding the requisite cellular and molecular mechanisms has made fear conditioning one of the most useful models for understanding learning from major perspectives: molecular, neural systems, and behavioral.

Such progress in understanding the basis of a rudimentary form of learning and memory may prove to be of great benefit to psychiatry. Other forms of learning are also fascinating, but there may be no other learning process more relevant to psychiatric disorders than amygdala-dependent fear learning. A full understanding of these mechanisms will surely have direct implications for clarifying the pathophysiology of and developing treatments for anxiety, phobia, panic, and posttraumatic stress disorders.

## WEB SITES OF INTEREST

http://www.pavlovian.org/
http://www.uvm.edu/~wfalls/
http://www.cns.nyu.edu/CNFA/
http://www.psych.nyu.edu/phelpslab/index.html
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*Accepted December 23, 2002.*

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DOI: 10.1097/01.CHI.0000046835.90931.32