I. CONDITIONED AND UNCONDITIONED FEAR

Since early 1980s, a great deal of progress has been made in delineating the neural pathways and the cellular and molecular mechanisms involved in fear, anxiety, and extinction of fear. Conditioned fear is a hypothetical construct used to explain the cluster of behavioral effects produced when an initially neutral stimulus is consistently paired with an aversive stimulus. For example, when a light that initially has no behavioral effect is paired with an aversive stimulus such as a foot shock, the light alone can now elicit a constellation of behaviors typically used to define a state of fear in animals. To explain these findings, it is generally assumed (cf. McAllister and McAllister, 1971) that during light-shock pairings (training session) the shock activates a central fear state that results in a variety of behaviors that can be used to infer a central state of fear (unconditioned responses — Fig. 12-1). After pairing, the light can now produce the same central fear state and thus the same set of behaviors formerly produced by the shock. Moreover, the behavioral effects that are produced in animals by this formerly neutral stimulus (now called a conditioned stimulus, CS) are similar in many respects to the constellation of behaviors used
FIGURE 12-1 Hypothetical description of unconditioned and conditioned fear. Prior to training, shock activates a hypothetical central fear state that produces a variety of behaviors that collectively define a state of unconditioned fear. Following cue—shock pairings, the cue can now activate the same hypothetical central fear state and hence produce a variety of behaviors that collectively define conditioned fear.

to diagnose generalized anxiety in humans. It is important to realize that the responses to the CS do not necessarily mimic those to the shock. For example, when a rat is given foot shock, the immediate reaction is to jump around to try to avoid the shock. However, when the rat is later presented with the CS, it does not jump around but, instead, freezes, quite the opposite of what the rat did during the shock. Thus the conditioned response in the case of fear conditioning often does not mimic the reaction to foot shock but, instead, the emotional effect of the foot shock. Treatments that block fear conditioning do not prevent the rat from jumping around but presumably do prevent the shock from having its usual emotional impact (Blair, Sotres-Bayon, Moita, and Ledoux, 2005; Miserendino, Sananes, Melia, and Davis, 1990).

II. FEAR VERSUS ANXIETY

Fear typically is very stimulus specific. Thus, a rat trained to fear a light by pairing it with a shock will not show a fear reaction to another stimulus, such as a tone or an odor. Fear comes on quickly and dissipates quickly. Thus, we react with fear when a snake crosses our path but get over our fear reaction
quickly once the snake is out of sight and we are sure it will not return. Anxiety has many of the same symptoms as fear; in fact, many of the laboratory measures of fear are identical to those used by psychiatrists to diagnose anxiety. However, even though the symptoms of fear and anxiety often are very similar, the stimuli or situations that elicit a state of anxiety often are not very specific. We feel anxious but are not quite sure what it is that bothers us. Anxiety is usually not abrupt in onset, like a state of fear, but instead comes on slowly and often lasts for a long time, in fact more or less continuously in certain patients.

**III. ANIMAL MODELS OF FEAR AND ANXIETY**

Table 12-1 lists a number of animal models of fear and anxiety. Those in boldface are the most widely used in the field. Although exact boundaries cannot always be established, tests in Section 1 of the table are primarily tests of stimulus-specific fear, typically after pairing a stimulus with a shock and then testing behavior in the presence of that stimulus, whereas those in Sections 2 and 3 are more like anxiety. These latter tests put the animal at “risk,” although the stimuli eliciting risk are not as predictable as those where a stimulus has consistently been paired with shock. For example, a 3-sec tone whose offset has consistently been paired with a foot shock elicits freezing, and this is clearly a test of conditioned fear. In contrast, a context that has been associated with shock is more like a test of anxiety because the animal is at “risk” when it reenters that context, although the exact time when a shock might occur is not predictable.

The two most widely used measures of conditioned fear are freezing and fear-potentiated startle (Fendt and Fanselow, 1999) and the systematic study of these behaviors by a host of investigators has rapidly led to a detailed understanding of the neural pathways and the cellular and molecular mechanisms of both the acquisition and expression of conditioned fear. Freezing is a species-typical response of rodents, to stop all behaviors, except for respiration, in the face of an imminent danger, such as the site of a predator or the presentation of a stimulus previously paired with shock, or placement in a context previously paired with shock. The freezing response is reliable, quantifiable, and easy and inexpensive to measure. The other major measure of conditioned fear is the fear-potentiated startle test. Because this is the test I happen to use and because it has certain advantages over the freezing measure, I use data gathered from this test to illustrate many of the basic principles and neural pathways involved in conditioned fear, because conclusions from fear-potentiated startle and freezing have largely, although not entirely, led to similar conclusions. At the end of this chapter I also discuss how different, but highly interconnected, brain structures are involved in fear versus anxiety, and finally I discuss selected
TABLE 12-1 Models of Fear and Anxiety in Rats

<table>
<thead>
<tr>
<th>Measures of fear and anxiety in animals</th>
<th>Possible human analogue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Tests measuring classically conditioned fear (response in presence of conditioned stimulus)</td>
<td></td>
</tr>
<tr>
<td>Freezing to CS or context paired with shock</td>
<td>Cessation of ongoing behavior</td>
</tr>
<tr>
<td>Fear-potentiated startle (more startle to loud sound in presence of conditioned stimulus — CS)</td>
<td>Increased startle</td>
</tr>
<tr>
<td>Conditioned emotional response (less bar pressing in presence of CS)</td>
<td>Cessation of ongoing behavior</td>
</tr>
<tr>
<td>Change in autonomic measures in presence of CS (heart rate, respiration, salivation, stomach acidity)</td>
<td>Heart pounding, panting, dry mouth, upset stomach</td>
</tr>
<tr>
<td>Tail flick test (rat takes longer time to move tail from hot light in presence of CS)</td>
<td>Stress or fear induced analgesia</td>
</tr>
<tr>
<td>Formalin test (rat takes longer time to lick paw in presence of CS)</td>
<td>Stress or fear induced analgesia</td>
</tr>
<tr>
<td>Active avoidance (animal makes response lever press, hurdle) to avoid shock in presence of CS</td>
<td>Avoidance of bad places</td>
</tr>
<tr>
<td>Inhibitory avoidance (animal does not return to place where shocked — used more as measure of memory)</td>
<td>Avoidance of bad places</td>
</tr>
<tr>
<td>Exposure to predator (rat freezes)</td>
<td>Fear of a lion</td>
</tr>
<tr>
<td>Electrical stimulation of central grey (rat escapes)</td>
<td>Panic???</td>
</tr>
<tr>
<td>Electrical stimulation of locus coeruleus (monkey grimaces, tries to escape, scans)</td>
<td>Scanning, vigilant behavior?</td>
</tr>
<tr>
<td>2. Tests requiring prior conditioning or deprivation to produce a non-zero baseline</td>
<td></td>
</tr>
<tr>
<td>Freezing to context paired with shock</td>
<td>Cessation of ongoing behavior</td>
</tr>
<tr>
<td>Operant conflict test (shock suppresses bar pressing)</td>
<td>Conflict (sex but then AIDS)</td>
</tr>
<tr>
<td>Lick suppression (shock suppresses lick rate)</td>
<td>Conflict</td>
</tr>
<tr>
<td>Negative contrast (press less for 4% solution after 32% compared to 4% all along)</td>
<td>Disappointment</td>
</tr>
<tr>
<td>Drug discrimination (rat presses pentylenetetrazol lever)</td>
<td>Pentylenetetrazol — induced anxiety</td>
</tr>
<tr>
<td>3. Tests not requiring conditioning</td>
<td></td>
</tr>
<tr>
<td>Elevated plus or X maze (rat stays in closed arms)</td>
<td>Fear of heights</td>
</tr>
<tr>
<td>Social interaction (rats don’t interact)</td>
<td>Social phobias</td>
</tr>
<tr>
<td>Light-dark box (rat goes to dark side)</td>
<td>Aversion to bright lights, open spaces</td>
</tr>
<tr>
<td>Open field test (rat stays near edge)</td>
<td>Aversion to large open spaces</td>
</tr>
<tr>
<td>Open field (rat grooms excessively)</td>
<td>Nail biting, fidgeting</td>
</tr>
<tr>
<td>Open field + food (hungry rat takes long time to eat)</td>
<td>Loss of appetite when afraid or anxious</td>
</tr>
<tr>
<td>Defensive withdrawal — open field test with “burrow” (tin can) (rat stays in tin can)</td>
<td>Hiding when afraid</td>
</tr>
<tr>
<td>Intraventricular infusion of CRF</td>
<td>Stress induced anxiety?</td>
</tr>
<tr>
<td>Light-enhanced startle</td>
<td>Aversion to bright lights</td>
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<tr>
<td>Ultrasonic vocalizations in rat pups separated from mother (rat emits 22–25 kHz sounds)</td>
<td>Crying as a result of separation</td>
</tr>
<tr>
<td>Urination, defecation following shock or in open field</td>
<td>Frequent urination, diarrhea</td>
</tr>
</tbody>
</table>
studies on extinction of conditioned fear and the clinical relevance of these findings.

IV. THE FEAR-POTENTIATED STARTLE EFFECT

Brown, Kalish, and Farber (1951) demonstrated that the amplitude of the acoustic startle reflex in the rat can be augmented by presenting the eliciting auditory startle stimulus in the presence of a cue (e.g., a light) that has previously been paired with a shock. This phenomenon, termed the fear-potentiated startle effect, has been replicated using either an auditory or a visual CS when startle is elicited by either a loud sound or an air puff (Davis, 1986). In this paradigm we typically pair a 3.7-sec light that coterminates with a 0.5-sec 0.4-mA shock. This is called the training session (Fig. 12-2). One to two days later, or a month or two if we are looking at remote memory, the rat is placed in a cage specially designed to measure the amplitude of the startle

![Figure 12-2](image)
reflex elicited by a burst of noise at the time the shock was presented in training [e.g., 3.2 sec after onset of the light (light-noise test trial) or in darkness (noise-alone)]. Conditioned fear is operationally defined by elevated startle amplitude in the presence of versus the absence of the cue previously paired with a shock (fear-potentiated startle — see Fig. 12-2). Thus, the CS does not elicit startle. Furthermore, the startle-eliciting stimulus is never paired with a shock; instead, the CS is paired with a shock and startle is elicited by another stimulus, in either the presence or the absence of the CS. Facilitation of a simple reflex is used to assay the hypothetical state of fear, which would be expected to facilitate reflexes. Fear-potentiated startle occurs only following paired versus unpaired or “random” presentations of the CS and the shock, which indicates that it is a valid measure of classical conditioning (Davis and Astrachan, 1978). Discriminations between visual and auditory conditioned stimuli (Davis, Hitchcock, and Rosen, 1987) or between auditory cues or visual cues that differ in duration (Davis, Schlesinger, and Sorenson, 1989; Siegel, 1967) have also been demonstrated with potentiated startle. Odors are especially good conditioned stimuli for fear-potentiated startle, in which reliable conditioning can be found after only a single pairing of the an odor with foot shock (Paschall and Davis, 2002). Increased startle in the presence of the CS still occurs very reliably at least one month after the original training, making it appropriate for the study of long-term memory as well (Campeau, Liang, and Davis, 1990). In fact, one of the major reasons that conditioned fear is used to investigate the neural mechanisms of memory is that fear lasts so long. Using freezing as a measure, Gale et al. (2004) reported that fear of a tone was still evident 16 months after original conditioning using rats, which have a life span of about 2–2.5 years. Fear-potentiated startle also can be measured in mice (Falls, Carlson, Turner, and Willott, 1997) and rhesus monkeys (Winslow, Parr, and Davis, 2002).

V. FEAR-POTENTIATED STARTLE IN HUMANS

Fear-potentiated startle can be seen in humans, using several different ways to elicit fear. In humans the eye-blink component of startle is the most easily measured and the most reliable because, although it habituates with repeated presentation of startle stimuli, it typically reaches a nonzero asymptote, so both excitatory and inhibitory effects can be measured. One way to potentiate startle in humans is via conditioning, using procedures that closely parallel those in rats (Grillon and Davis, 1997; Hamm, Greenwald, Bradley, Cuthbert, and Lang, 1991; Hamm, Start, and Vaitl, 1990; Lipp, Sheridan, and Siddle, 1994). For example, Christian Grillon and I (Grillon and Davis, 1997) presented undergraduates with a light consistently paired with a shock (paired group), a light explicitly unpaired with a shock (unpaired group), or a light
that served as a signal to push a button as soon as a second light came on
(reaction time group). Startle was measured in the presence or absence of these
lights prior to and following conditioning and then measured again 1 week
later. In the paired group, startle magnitude was greater in the presence versus
the absence of the light in both the first and second sessions, indicating reten­
tion of conditioning over a 1-week interval. Startle was not elevated when
elicited in the presence of the light in either session in the unpaired group or
in the reaction time group. This indicates that fear-potentiated startle only
occurred following explicit pairing of a cue with a shock and was not simply
a function of heightened arousal following shock presentation or instructions
to perform in a reaction time experiment, consistent with earlier work (Hamm,
Start, and Vaitl, 1990; Lipp, Sheridan, and Siddle, 1994). Interestingly, these
earlier studies showed that arousal associated with either shock or with a reac­
tion time experiment increased the galvanic skin response, a measure of activa­
tion of the sympathetic nervous system. Thus changes in startle reflected a
change in valence (threat versus safe) and arousal, whereas the galvanic skin
response did not differentiate between valence and arousal. Finally, in Session
2 there was a pronounced increase in startle amplitude in the beginning of the
session in the unpaired group but not in the paired group, indicative of context
conditioning, a result predicted from contemporary learning theory (Rescorla
and Wagner, 1972). Thus, fear-potentiated startle measured using conditioning
procedures in humans closely parallels work done in rodents.

Another way to potentiate startle is simply to tell people that when a
certain colored light comes on they might get a shock (Grillon, Ameli, Woods,
Merikangas, and Davis, 1991). Thus, even though they have never actually
received a shock, just the anticipation of this possibility, which is rated to be
very fearful, is enough to increase startle magnitude in humans. Finally, startle
elicited in the presence of pictures of scary scenes, such as a snake or dog ready
to attack, is potentiated as compared to when it is elicited in the presence of
neutral pictures, such as baskets or cans (Lang, Bradley, and Cuthbert, 1990).
In contrast, startle is actually inhibited when elicited in the presence of pleasant
pictures, such a babies or sexy scenes, whereas the galvanic skin response is
increased in the presence of both scary and pleasant scenes. Once again, there­
fore, startle is sensitive to valence but not simply arousal (Lang, Bradley, and

VI. NEURAL PATHWAYS INVOLVED IN FEAR-
POTENTIATED STARTLE

One of the major advantages of the fear-potentiated startle test is that the
hypothetical state of fear is inferred from an increase in a simple reflex. More­
over, because the acoustic startle reflex has such a short latency (e.g., 8 msec
FIGURE 12-3  Schematic diagram of the primary acoustic startle reflex pathway. Axons from sensory receptors in the cochlea synapse onto cochlear root neurons (CRNs) embedded in the auditory nerve. CRNs project to a ventrolateral region of the nucleus reticularis pontis caudalis (PnC). PnC axons form the reticulospinal tract make mono- and polysynaptic connections in the spinal cord onto motoneurons that innervate muscles in the neck, forelimbs, and hind limbs that mediate the acoustic startle reflex.

measured electromyographically in the hind leg, 5 msec in the neck), it must be mediated by a simple neural pathway. We now believe that the primary acoustic startle reflex pathway involves three central synapses: (1) auditory nerves fibers to cochlear root neurons (CRNs) (2), CRN axons to cells in the nucleus reticularis pontis caudalis (PnC), and (3) PnC axons to motor neurons in the facial motor nucleus (pinna reflex) or spinal cord (whole body startle — Fig. 12-3).

A. Cochlear Root Neurons

In rats there is a small group (about 20 on each side) of very large cells (35 μm in diameter) embedded in the cochlear nerve, called cochlear root neurons. These
neurons receive direct input from the spiral ganglion cells in the cochlea, making them the first acoustic neurons in the central nervous system (Lopez, Merchan, Bajo, and Saldana, 1993). They send exceedingly thick axons (sometimes as wide as 7 μm) through the trapezoid body, at the very base of the brain, to the contralateral side, to an area just medial and ventral to the lateral lemniscus, and continue on up to the deep layers of the superior colliculus. However, they give off thick axon collaterals that terminate directly in the PnC (Lingenhohl and Friauf, 1994; Lopez, Merchan, Bajo, and Saldana, 1993), exactly at the level known to be critical for the acoustic startle reflex (cf. Lee, Lopez, Meloni, and Davis, 1996). Nodal and Lopez (2003) showed direct connections between CRN axons to cells in the PnC that projected to the spinal cord, based on double-labeling techniques. Electron microscopy of the labeled CRNs axons and terminals showed that even the thinnest processes were myelinated, consistent with very rapid transmission. Multiple CRNs synapse onto single reticulospinal neurons in PnC, where most of the connections were axodendritic, with multiple asymmetric synapses, consistent with an excitatory input.

Bilateral chemical lesions of the cochlear root neurons essentially eliminate acoustic startle in rats, and the magnitude of decrease in startle was highly correlated with the number of CRNs destroyed (Lee, Lopez, Meloni, and Davis, 1996). Although damage to the auditory root, where the cochlear root neurons reside, has not been fully ruled out, other tests indicated that these animals could clearly orient to auditory stimuli (e.g., suppression of licking) and had normal compound action potentials recorded from the cochlear nucleus (Lee, Lopez, Meloni, and Davis, 1996).

**B. Nucleus Reticularis Pontis Caudalis (PnC)**

Very discrete N-methyl-D-aspartate (NMDA)-induced lesions of cell bodies in the PnC completely eliminated startle, whereas NMDA-induced lesions of the ventral nucleus of the lateral lemniscus or the area just ventral and medial to it did not, provided the lesion did not extend to the PnC (Lee, Lopez, Meloni, and Davis, 1996). Local infusion of the NMDA antagonist D-2-amino-5-phosphono-pentanoic acid (AP5) into the PnC reduced startle by 80–90% (Miserendino and Davis, 1993), at doses 1/60 of those that depressed startle after infusion into the area of the ventral lateral lemniscus (Spiera and Davis, 1988). Moreover, comparably low doses of the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) also depressed startle after local infusion into the PnC (Miserendino and Davis, 1993) but had no effect when infused into the area of the ventral lateral lemniscus, even using much higher doses (Lee and Davis, unpublished). Single-pulse electrical stimulation of the PnC elicited startle responses, with a latency of about 5 msec recorded in the hind
leg, compared to about 8 msec when elicited acoustically (Davis, Gendelman, Tischler, and Gendelman, 1982). In humans, startle stimuli lead to an increase in blood flow in the PnC, and this effect habituates with repetition of the startle stimulus (Pissiota, Frans, Fredrikson, Langstrom, and Flaten, 2002).

C. Facial and Spinal Motor Neurons

In rats the pinna component of the startle reflex consists of a rapid backward movement of the pinna, which covers and protects the ear, and the pinna reflex shows many of the features of whole-body startle, including fear-potentiated startle (Cassella and Davis, 1986). The motor neurons that innervate the relevant pinna muscles are located in the dorsolateral division of the facial motor nucleus to which the PnC has direct projections. Startle stimuli elicit action potentials in facial motor nucleus neurons, with a latency of 5 msec (Cassella and Davis, 1987) prior to movement of the pinna muscles, and local infusion of the AMPA/kainite antagonist CNQX into the facial motor nucleus eliminated the click-elicited pinna reflex on the ipsilateral but not the contralateral side (Meloni and Davis, unpublished observations).

Motor neurons in the lumbar spinal cord innervate muscles in the hind leg that provide the major extension/flexion component of startle in rodents (Davis, 1984). When startle is measured electromyographically (EMG) in the hind leg, two distinct components can be measured, a short latency component (~8 msec) and a slightly longer latency component (~15 msec). Infusion in the space between the spinal cord and the membranes that surround the spinal cord (intrathecal infusion) of the AMPA/kainate antagonist CNQX in the vicinity of the lumbar motor neurons eliminated the short latency component but not the longer latency component, whereas infusion of the NMDA antagonist AP5 had just the opposite effect (Boulis, Kehne, Miserendino, and Davis, 1990). Infusion of both compounds together totally eliminated the EMG component of startle in the hind leg. This suggests that the acoustic startle reflex involves motor neurons in the spinal cord that are activated by release of glutamate acting on both non-NMDA and NMDA receptors. Intrathecal administration of cAMP or cAMP analogues markedly facilitate acoustic startle amplitude (Boulis and Davis, 1990; Kehne, Astrachan, Astrachan, Tallman, and Davis, 1986), probably by increasing the release of glutamate from the terminals of neurons in the PnC activated by the startle stimulus. Intrathecal administration of the glycine receptor antagonist strychnine markedly increases startle amplitude (Kehne, Gallager, and Davis, 1981), as do norepinephrine (Davis, Astrachan, Kehne, Commissaris, and Gallager, 1984) and serotonin agonists (Davis, Astrachan, Gendelman, and Gendelman, 1980; Davis, Astrachan, Kehne, Commissaris, and Gallager, 1984), which are known to facilitate the response of motor neurons to glutamate.
VII. FEAR-POTENTIATED STARTLE MEASURED ELECTROMYOGRAPHICALLY

Having delineated what we believe is the primary acoustic startle pathway, we hoped to use this information to deduce where fear ultimately alters neural transmission so as to increase acoustic startle amplitude. Because startle can be measured with a latency of only 8 msec, the light should potentiate this 8-msec response. Typically, however, startle is not measured electromyographically, but instead it is measured as a movement of a cage over a relatively long interval after onset of the startle-eliciting stimulus (e.g., 200 msec). Hence it is possible that the visual CS does not actually alter the very short-latency startle response, but instead it might facilitate transmission in other auditory systems, which could produce cage movements at longer latencies. If so, this might mean that the visual CS would not actually alter transmission along the short-latency pathway outlined in Figure 12-3. However, if the light did increase the very short short-latency startle reflex, we would have to conclude that it alters transmission at some point in the short-latency pathway. In fact, we found that a light previously paired with a foot shock markedly potentiated the short-latency startle response, measured electromyographically in the neck muscles (Fig. 12-4 Cassella, Harty, and Davis, 1986) indicating that the visual CS must ultimately alter neural transmission somewhere along the short-latency pathway outlined in Figure 12-3.

VIII. THE POINT IN THE STARTLE PATHWAY WHERE FEAR MODULATES TRANSMISSION

Having demonstrated that fear facilitates transmission in this very short-latency pathway, the next task was to try to deduce where a light previously paired with a shock ultimately modulates transmission in this short-latency pathway. We had previously shown that startle could be elicited with single electrical pulses at various points along the startle pathway, with progressively shorter latencies as the electrode was moved from the cochlear root axons to the reticulospinal axons connecting the PnC with spinal motor neurons (Davis, Gendelman, Tischler, and Gendelman, 1982), and used this method to deduce where habituation and sensitization occurred within the startle pathway (Davis, Parisi, Gendelman, Tischler, and Kehne, 1982). The logic of this approach to try to determine where fear ultimately might alter transmission in the startle pathway is shown in Figure 12-5. Let us assume that the light, after being paired with a shock, goes from the retina and, in one way or another, ultimately modulates transmission in the PnC to increase acoustic startle amplitude. If one were to elicit startle acoustically or electrically by eliciting startle from points in the pathway upstream from the PnC, then both acoustically
and electrically elicited startle should be facilitated by the light because the startle signal would have to pass through the PnC. On the other hand, if startle were elicited electrically from points downstream from the PnC, it would not be increased in the presence of the light because the startle signal would not pass through the PnC. Although this logic requires a number of assumptions, this is exactly what we found (Berg and Davis, 1985). Thus, startle elicited electrically from CRN axons adjacent to the ventral cochlear nucleus or farther along the base of the brain on route to the PnC was facilitated by the light, whereas startle elicited in the PnC or the reticulospinal tract was not, even though acoustically elicited startle was increased in both cases. Systemic administration of diazepam (Valium), which reduces fear and anxiety in people,
selectively decreased fear-potentiated startle elicited electrically from points afferent to the PnC, indicating that eliciting startle in this way could pick up the anxiolytic effect of diazepam (Berg and Davis, 1984).

**IX. PROJECTIONS TO THE PNC**

Having determined that the PnC was the probable site where fear ultimately altered transmission to increase startle amplitude, we posed the next question: What parts of the brain project to the part of the PnC critical for startle, and are these projections critical for fear-potentiated startle. After several years now, we believe that there are three parallel pathways, each of which may play a part in fear-potentiated startle.

**A. Direct Projections from the Central Nucleus of the Amygdala**

Local infusion of the retrograde tracer FluoroGold into the part of the PnC critical for startle resulted in labeling of neurons in the medial division of the central nucleus of the amygdala (Rosen, Hitchcock, Sananes, Miserendino, and
Davis, 1991). This was an exciting finding because earlier work in several laboratories (Blanchard and Blanchard, 1972; Gentile, Jarrel, Teich, McCabe, and Schneiderman, 1986; Iwata, LeDoux, Meeley, Arneric, and Reis, 1986; Kapp, Frysinger, Gallagher, and Haselton, 1979) as well as our own (Hitchcock and Davis, 1986, 1987) had implicated the central nucleus of the amygdala in conditioned and unconditioned fear using several different measures. Local infusion into the central nucleus of the amygdala of an anterograde tracer confirmed this connection and was used to delineate the course of the pathway from the central nucleus of the amygdala to the PnC (Fig. 12-6). Electrolytic lesions at various points along this pathway blocked fear-potentiated startle but had no effect on baseline startle amplitude (Hitchcock and Davis, 1991). In contrast, electrolytic lesions of outputs of the central nucleus of the amygdala to the bed nucleus of the stria terminalis had no effect on fear-potentiated startle, consistent with earlier work (LeDoux, Iwata, Cicchetti, and Reis, 1988).

B. Indirect Projections from the Central Nucleus of the Amygdala via the Deep Mesencephalic Reticular Formation

Although these results with electrolytic lesions were consistent with idea that this direct projection mediates fear-potentiated startle, it is still possible that synaptic, rather than direct, projections might also be involved. For example, injection of a retrograde tracer into the PnC showed that several nuclei that lie along this direct pathway contained neurons that also projected directly to the PnC. One of the most prominent of these was in the mesencephalic reticular formation and deep layers of the superior colliculus (deep SC/DpMe). The amygdala sends heavy, broad projections to this part of the rostral midbrain (Rosen, Hitchcock, Sananes, Miserendino, and Davis, 1991), which in turn projects to the PnC (Cameron, Iqbal, Westlund, and Willis, 1995; Meloni and Davis, 1999). Collision tests using electrical brain stimulation suggested that a synapse existed between the amygdala and the midbrain, and electrolytic lesions in the midbrain blocked fear-potentiated startle (Yeomans and Pollard, 1993). Thus, the rostral midbrain was proposed to be a relay between the amygdala and the PnC in fear-potentiated startle (Yeomans and Pollard, 1993).

Consistent with this hypothesis, inactivation of the deep layers of the superior colliculus/the deep mesencephalic nucleus (deep SC/DpMe) with muscimol blocked the expression but not the acquisition of fear-potentiated startle (Meloni and Davis, 1999), suggesting an effect on the output circuitry of the amygdala rather than a blockade of sensory input to the amygdala. Although these results confirmed a critical relay in the midbrain in mediating fear-potentiated startle, the precise part of the midbrain remained unclear. Recently
FIGURE 12-6  Schematic diagram of output of the central nucleus of the amygdala, rostrally to the bed nucleus of the stria terminalis and then caudally down the ventral amygdalofugal tract (VAF), ultimately terminating in the nucleus reticularis pontis caudalis (PnC).
we found that local infusion of the AMPA/Kainate glutamatergic receptor antagonist NBQX [2,3-dihydroxy-6-nitro-7-sulphamoylbenzo(F)-quinoxaline], which has a very limited area of diffusion (Walker, Paschall, and Davis, 2005), blocked the expression but not the acquisition of fear-potentiated startle if infused into the deep SC/DpMe (Zhao and Davis, 2004). In contrast, infusion of the same doses, either 1 mm lateral into the lateral mesencephalic reticular formation or 1 mm medial into the dorsal/lateral periaqueductal gray or into the superficial layers of the superior colliculus had no effect. The infusions altered not the baseline startle response but only startle facilitated by a conditioned fear stimulus. These data suggest that fear-potentiated startle is mediated by release of glutamate into the deep SC/DpMe and support the idea of an indirect amygdalo–tecto–PnC pathway in which the rostral midbrain serves as a relay between the amygdala and the PnC to mediate fear-potentiated startle (Yeomans and Pollard, 1993).

C. Indirect Projections from the Medial Nucleus of the Amygdala via the Ventromedial Hypothalamus and Ventral Periaqueductal Gray

Despite clear evidence that the medial nucleus of the amygdala is involved in sexual behavior in both males and females, a growing body of literature points to a role of the medial nucleus of the amygdala in stress, especially so-called psychological stress. For example, Dayas et al. (2001) found that psychological stressors such as noise, restraint, and forced swim elicited high levels of c-fos expression in the medial nucleus of the amygdala as compared to the central nucleus of the amygdala, whereas physical stressors such as hemorrhage and immune challenge produced the opposite pattern of c-fos expression. The medial nucleus of the amygdala and its outputs to the hypothalamus and periaqueductal gray have been implicated in defensive behavior in cats (Adamec, 1994), and we recently found that blockade of AMPA/kainate glutamate receptors in the medial nucleus of the amygdala blocked conditioned fear elicited not only by an odor but also by a light previously paired with foot shock (Walker, Paschall, and Davis, 2005).

In a series of studies we have found that local infusion into the medial nucleus of the amygdala of either morphine (Davis, Yong, Shi, and Zhao, in preparation) or substance P antagonists (Zhao, Yong, and Davis, in preparation) blocked the expression of fear-potentiated startle, without any effect on baseline startle amplitude. The medial nucleus of the amygdala sends heavy projections to the ventral medial hypothalamus, and local infusion of either NBQX, morphine, or substance P antagonists into this region also totally blocked fear-potentiated startle, without any effect on baseline startle amplitude. Although the ventral medial hypothalamus does not project directly to the PnC, it does
project to the periaqueductal gray, which in turn projects to the PnC. Recall, however, that local infusion of NBQX into the periaqueductal gray did not block the expression of fear-potentiated startle. Nonetheless, local infusion of either morphine or Substance P antagonists into the periaqueductal gray did block fear-potentiated startle. This suggests that the fear-potentiated startle may be mediated or importantly modulated by the release of Substance P in the medial nucleus of the amygdala, ventral medial hypothalamus, and the periaqueductal gray. The effect of morphine at each of these areas might be due to its ability to decrease the release of Substance P by acting on terminal mu opioid autoreceptors.

Thus far the effects of Substance P antagonists into the PnC have not been tested on fear-potentiated startle. However, Substance P increases the responsiveness of reticulospinal neurons to acoustic stimuli (Krase, Koch, and Schnitzler, 1994), and local infusion into the PnC of a Substance P antagonist completely blocked the normal sensitizing effect of foot shock on startle (Krase, Koch, and Schnitzler, 1994), which has previously been deduced ultimately to modulate transmission at the level of the PnC (Boulis and Davis, 1989). Because Substance P is positively coupled to cAMP in some brain areas (Mitsuhashi, Osashi, Shichijo, Christian, Sudduth-Klinger, Harrowe, and Payan, 1992), it is possible that it acts in the PnC via activation of cAMP.

Thus, there appear to be three parallel routes whereby the amygdala can modulate startle during a state of conditioned fear: (1) a direct pathway from the central nucleus of the amygdala to the PnC; (2) an indirect pathway from the central nucleus of the amygdala to the deep SC/Me to the PnC, where glutamate acting on AMPA/kainate receptors seems to be critical; and (3) an indirect pathway from the medial nucleus of the amygdala to the ventral medial hypothalamus to the periaqueductal gray to the PnC, where Substance P receptors seem to be critical (Fig. 12-7).

**X. ROLE OF THE AMYGDALA IN FEAR**

I have just given you a detailed description for how fear modulates a simple reflex in terms of the neural circuitry involved in the reflex, using the acoustic startle reflex as an example, and the way in which the amygdala connects to the reflex pathway. Moreover, this is just one example of many showing that outputs of the central nucleus of the amygdala to the hypothalamus and brainstem are involved in many of the specific signs of fear and anxiety, as illustrated in Figure 12-8. However, this is only the "output" side of the story. One still needs to explain how the sensory stimuli, including foot shocks, activate the amygdala and how pairing sensory stimuli with foot shock can produce a "memory" that can last for a very long time.
A. Anatomy of the Amygdala — Intrinsic Connections

The amygdala complex is a complicated group of interconnected nuclei, each of which comprises several subdivisions. In the rodent the amygdala generally has been divided into the basolateral amygdala, which includes the lateral, basal, and accessory basal nuclei, and several structures surrounding the basolateral amygdala, including the central, medial, and cortical nuclei.

1. Lateral Nucleus

The lateral nucleus comprises three subdivisions: the dorsolateral, ventrolateral, and medial divisions. The dorsolateral nuclei receives both thalamic and cortical input and projects to the medial division, which also receives input from multisensory cortical processing regions, including the prefrontal and perirhinal cortex (Pitkanen, Savander, and LeDoux, 1997). Thus, the medial division of the lateral nucleus receives sensory information via thalamic and cortical inputs, some of which is processed in the dorsolateral division, and sends outputs to other parts of the amygdala, which include the accessory basal nucleus, the basal nucleus, the periamygdaloid cortex, the medial nucleus, the
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Light, tone, smell, touch

Shock

Basolateral Amygdala

Explicit Cues
Short Term
Conditioned
Highly Predictable

Lateral Hypothalamus
Dorsal Motor N. Vagus
N. Ambiguus
Parabrachial N.
Ventral Tegmental N.
Locus Coeruleus
Dorsal Lat. Tegmental N.
N. Basalis (Forebrain)
Reticular Formation
Paraventricular N.
Facial, Trigeminal N.
Central Gray

Anatomical Target

Signs, Symptoms of Fear or Anxiety

HR, GSR, BP, pale, pupils open
Ulcers, urination, defecation, HR
BP, vocalization
Panting, respiratory distress
Behavioral and EEG arousal
Increased vigilance
Increased attention
Increased motor responses
Reflex facilitation
HPA axis activation
Facial expression, open mouth
Freezing, hypoalgesia,
vocalization

Fear

FIGURE 12-8 Schematic diagram of the outputs of the central nucleus to various hypothalamic and brainstem targets and how these targets are involved in specific signs of fear.

posterior cortical nucleus, the capsular division of the central nucleus, and the lateral division of the amygdalohippocampal area (Pitkanen, Stefanacci, Farb, Go, LeDoux, and Amaral, 1995).

2. Basal and Accessory Basal Nuclei

The basal amygdala comprises the magnocellular, intermediate, and parvicellular divisions (Savander, Go, LeDoux, and Pitkanen, 1995). The parvicellular division is the source of most of the projections within the basal nucleus, projecting to both the magnocellular and intermediate divisions. The main projections from the basal nucleus to other parts of the amygdala include the lateral olfactory tract, the anterior amygdaloid area, the medial and capsular divisions of the central nucleus, the anterior cortical nucleus, and the amygdalohippocampal area (Savander, Go, LeDoux, and Pitkanen, 1995). In addition, the magnocellular and intermediate divisions of the basal nucleus send heavy projections to homonymous regions of the amygdala on the contralateral side. The accessory basal nucleus sends projections to the medial and capsular divisions of the central nucleus, the medial division of the amygdalohippocampal area, the medial division of the lateral nucleus, the central division of the medial nucleus, and the posterior cortical nucleus (Savander, Go, Ledoux, and
Thus, outputs from the lateral nucleus can affect large parts of the amygdala via relays in the basal and accessory basal nucleus.

### 3. Central Nucleus of the Amygdala

The central nucleus receives substantial inputs from most of the amygdaloid nuclei, including the lateral, basal, accessory basal, and anterior cortical nuclei, as well as the nucleus of the lateral olfactory tract, the periamygdaloid cortex, and the intercalated nuclei (cf. Jolkkonen and Pitkanen, 1998). In the rat, the central nucleus of the amygdala comprises four major subdivisions: the medial, lateral, lateral capsular, and the intermediate subdivisions (McDonald, 1982). Inputs from the amygdaloid and extra-amygdaloid areas terminate in various divisions of the central nucleus. The capsular division of the central nucleus receives input from the lateral, basal, and accessory basal nuclei, and the medial division gets input from the basal nucleus and accessory basal nuclei. The lateral division of the central nucleus projects to the capsular and medial divisions (Jolkkonen and Pitkanen, 1998). The intermediate division does not seem to project to any of the other divisions of the central nucleus. Interestingly, the lateral division of the central nucleus receives input from other amygdala nuclei but also gets direct input from several cortical areas (Jolkkonen and Pitkanen, 1998).

### B. Inputs into the Amygdala Relevant for Fear Conditioning

The amygdala receives input from numerous areas of the brain, many of which are critical for fear conditioning.

#### 1. Pain

Because fear conditioning almost always is produced by pairing some sensory stimulus with pain, the emotional aspect of which becomes the conditioned response, I begin by describing how pathways providing pain information get to the amygdala and the relevance of these pathways for fear conditioning (Fig. 12-7). During fear conditioning, foot-shock information is transmitted to the amygdala via parallel pathways that include the posterior intralaminar nuclei in the thalamus and the parietal insular cortex. Besides receiving acoustic inputs from the inferior colliculus, the posterior intralaminar nuclei also receive somatic pain inputs from the spinal cord and in turn project to the amygdala, particularly the lateral amygdaloid nucleus (cf. Shi and Davis, 1999). Electrical stimulation of this area is an effective unconditioned stimulus for fear conditioning, similar to foot shock (Cruikshank, Edeline, and Weinberger, 1992). Thus, this thalamo-amygdaloid pathway may serve as the unconditioned
stimulus pathway during emotional learning. However, pretraining lesions of the posterior intralaminar nuclei alone did not prevent the acquisition of fear conditioning (Campeau and Davis, 1995b; Romanski and LeDoux, 1992b), indicating that additional pathway(s) must contribute foot-shock information to the amygdala.

The caudal part of insular cortex, the so called parietal insula, receives convergent inputs from somatosensory cortices, ventroposterior and posterior thalamic nuclei, posterior intralaminar nuclei, and the midbrain parabrachial nucleus (cf. Shi and Davis, 1999). Further, this portion of the insular cortex is probably a primary source in providing cortical somatosensory information to the amygdala. Both the parietal insular cortex and posterior intralaminar nuclei of thalamus in turn project to the lateral, basolateral, basomedial, and central nuclei of the amygdala.

Consistent with this, combined lesions of both parietal insular cortex and posterior intralaminar nuclei of the thalamus were necessary to interrupt the transmission of foot-shock information to the amygdala and thus block the acquisition of fear-potentiated startle (Shi and Davis, 1999). Importantly, however, these lesions did not block the expression of fear-potentiated startle once conditioning had taken place, as one would expect if these pathways were involved in fear acquisition. These combined lesions also reduced the degree to which rats reacted to footshock, which we believe is modulated by the amygdala. Thus, even though the major immediate reactions of a rat to foot shock involve brainstem and spinal cord reflex circuits, these reflexes are most probably modulated by the amygdala, just as the startle reflex is, which also is mediated by a brainstem and spinal cord reflex circuit. Hence lesions that interrupt shock inputs to the amygdala would be expected to influence shock reactivity. This may explain why chemical lesions in some of these thalamic nuclei failed to block fear conditioning, because they also failed to alter shock reactivity (Brunzell and Kim, 2001). We believe the difference was due not to the use of chemical lesions, but, rather, to incomplete lesions, because we have found that complete chemically induced lesions of the posterior intralaminar nuclei block acquisition of fear-potentiated startle and also reduce shock reactivity (Shi and Davis, unpublished observations).

2. Hearing

A great deal of work has been done using auditory cues to study the role of the amygdala in fear conditioning, as exemplified by the elegant work in Dr. Joseph LeDoux’s laboratory. Auditory inputs from modality-specific areas of thalamus and cortex exclusively or primarily target the dorsolateral and ventrolateral divisions of the lateral amygdaloid nucleus (cf. Romanski, Clugnet, Bordi, and LeDoux, 1993), and single-unit recording studies find that cells in the dorsolateral division fire with the shortest latencies (12–25 msec) to an
auditory stimulus (Bordi and LeDoux, 1992). This division also receives input from somatosensory areas activated by foot shock (Romanski, Clugnet, Bordi, and LeDoux, 1993), and the firing rate of cells activated at short latencies (mean ~25 msec) is increased when these tones are paired with foot shocks (Quirk, Repa, and LeDoux, 1995). Both electrolytic and excitotoxic posttraining lesions of the lateral nucleus of the amygdala, sparing a large number of basolateral neurons, disrupted fear-potentiated startle to both auditory and visual conditioned stimuli (Campeau and Davis, 1995a), consistent with earlier work using auditory cues, pretraining lesions, and freezing as the measure of fear (LeDoux, Cicchetti, Xagoraris, and Romanski, 1990). All auditory inputs to the lateral nucleus of the amygdala ultimately arise from the auditory thalamus, and complete electrolytic or excitotoxic lesions of the entire auditory thalamus specifically disrupted fear-potentiated startle to an auditory but not a visual CS, whether the lesions were made before or after conditioning (Campeau and Davis, 1995b), consistent with earlier work using pretraining lesions and freezing (LeDoux, Sakaguchi, Iwata, and Reis, 1986; LeDoux, Sakaguchi, and Reis, 1984).

Although it has been argued that the direct projection from the thalamus to the amygdala is critical for conditioned fear to an auditory stimulus (Romanski and LeDoux, 1992b), this conclusion is based on results where lesions of a given pathway are made prior to fear conditioning. When lesions are made after fear conditioning, we find that the subcortical pathway probably is not normally used but instead can take over if the thalamo-cortical pathway is disrupted. The cortical pathway I am referring to is not the primary auditory cortex but instead a secondary multisensory cortex called the perirhinal cortex. These conclusions are based on the following observations.

Lesions of the ventral and dorsal divisions of the medial geniculate body, giving rise to the main thalamo-cortico-amygdala pathway, significantly disrupted fear-potentiated startle to an auditory but not a visual CS (Campeau and Davis, 1995b). In contrast, animals with posterior thalamic lesions, which project directly to the lateral nucleus of the amygdala, actually had higher levels of fear-potentiated startle, especially to the auditory CS. However, the subcortical pathway can be recruited to mediate fear-potentiated startle to an auditory conditioned stimulus when animals sustaining ventral and dorsal medial geniculate body lesions were retrained. This could explain why lesions of the cortical pathway made prior to fear conditioning, such as those done in the LeDoux lab, would not disrupt conditioned fear, because under these circumstances the subcortical pathway would take over.

Neither pre- nor posttraining auditory cortex ablations, mostly restricted to the primary auditory area, had a reliable effects on fear-potentiated startle (Campeau and Davis, 1995b). In contrast, posttraining lesions to the secondary auditory and perirhinal cortices completely blocked fear-potentiated startle to both auditory and visual CSs, but, importantly, pretraining lesions did not
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reliably affect fear-potentiated startle to either CS. The posttraining deficits were observed only after the lesions included most of the rostral-caudal extent of the perirhinal area, which also receives visual input. The differences observed between pre- and posttraining lesions did not arise from differences in lesion size, because the extent of the lesions in each study was similar. These results are consistent with the findings of LeDoux’s lab (Romanski and LeDoux, 1992a, 1992b) showing that pretraining perirhinal area lesions do not reliably disrupt conditioned fear response to an auditory stimulus as well as with those of our lab (Rosen, Hitchcock, Miserendino, Falls, Campeau, and Davis, 1992) showing that posttraining lesions disrupt fear-potentiated startle to a visual stimulus. Thus, by using posttraining lesions we conclude that the pathway going from the auditory thalamus to the perirhinal cortex to the lateral nucleus of the amygdala is normally used in fear-potentiated startle when an auditory CS is used. The difference between this conclusion and earlier ones reflects the use of pre- versus posttraining lesions and has been confirmed by later work in Ledoux’s lab using posttraining lesions of the perirhinal cortex (Corodimas and LeDoux, 1995).

3. Vision

Like the auditory system, the use of posttraining lesions has led us to conclude that subcortical projections from the visual thalamus (e.g., the lateral posterior nucleus) to the amygdala are not normally used in fear-potentiated startle using a visual CS. Instead, we believe that projections from the lateral posterior nucleus of the thalamus to the perirhinal cortex and then into the amygdala are the ones normally used. This is based on the following evidence. Lesions or chemical inactivation of superficial layers of superior colliculus, which receive massive retinal input, do not disrupt the expression of fear-potentiated startle to a visual CS (Meloni and Davis, 1999; Tischler and Davis, 1983). The lateral posterior nucleus of the thalamus also receives direct projections from the retina, and anterograde anatomical tract-tracing studies in our laboratory (Shi and Davis, 2001) showed it sends heavy projections to area TE2 and the dorsal perirhinal cortex and moderate projections to the lateral amygdaloid nucleus. However, posttraining lesions restricted to the lateral posterior nucleus did not block the expression of fear conditioning using a visual CS. Posttraining lesions of neither the dorsal lateral geniculate nucleus (Shi and Davis, 2001), which receives retinal inputs, nor the visual cortex, including V1 and V2, prevented the expression of conditioned fear responses using a visual CS (Falls and Davis, 1994; LeDoux, Romanski, and Xagoraris, 1989; Rosen, Hitchcock, Miserendino, Falls, Campeau, and Davis, 1992; Tischler and Davis, 1983).

However, both Te2 and the perirhinal cortex receive visual inputs from lateral posterior nucleus (Shi and Davis, 2001) and visual cortices and in turn
project to the amygdala, and combined lesions of both dorsal lateral geniculate nucleus and lateral posterior nucleus, which would cut off both thalamic and cortical routes to Te2 and perirhinal cortex, totally blocked the expression of conditioned fear using a visual CS. Local infusion of the glutamate antagonist NBQX had the same effect, suggesting that the lesion effect did not result from damage to fibers of passage. Based on these and other results, we conclude that visual input carried by projections from the dorsal lateral geniculate nucleus and lateral posterior nucleus via connections through Te2 and perirhinal cortex to the amygdala normally are involved in conditioned fear using a visual CS.

4. Smell

Olfactory stimuli are unique because olfactory receptors in the nose send axons to the olfactory bulb, which then projects directly to the corticomedial nucleus of the amygdala (i.e., one synapse between the receptor and the amygdala). The olfactory bulb also projects to the piriform cortex, which then projects to the basolateral nucleus of the amygdala. As mentioned earlier, inactivation of the medial nucleus of the amygdala blocks the expression of fear-potentiated startle using either an olfactory or a visual CS. However, as is discussed shortly, NMDA antagonists infused into the basolateral nucleus of the amygdala block the acquisition of conditioned fear, and this is also true for fear-potentiated startle using olfactory cues as conditioned stimuli (Walker, Paschall, and Davis, 2005). However, infusion of NMDA antagonists into the medial nucleus of the amygdala does not block the acquisition of fear-potentiated startle, suggesting that it is more on the output than on the input side in terms of conditioned fear using olfactory cues. Thus, the more indirect pathway from the olfactory bulb to the piriform cortex to the basolateral nucleus of the amygdala is probably the route necessary for conditioned fear, and both pre- and posttraining lesions of the basolateral nucleus of the amygdala blocked fear conditioning to olfactory cues. Finally, like fear conditioning to visual or auditory cues (see later), the perirhinal cortex, to which the piriform cortex projects, may also be important for fear conditioning using olfactory cues (Herzog and Otto, 1997; Schettino and Otto, 2001), although we have not looked at its role or the role of piriform cortex in olfactory mediated fear potentiated startle.

C. Plasticity in the Amygdala During Fear-Potentiated Startle Training

I have now described how the amygdala receives sensory input from several modalities along with shock input known to be critical for fear conditioning. I have also used the fear-potentiated startle effect to illustrate the role of these
various sensory pathways in the acquisition and expression of fear-potentiated startle and how parallel outputs from the central and medial nuclei of the amygdala connect to the startle pathway, which, in one way or another, increases acoustic startle amplitude at the level of the PnC in the presence of a conditioned fear stimulus. However, it remains to be determined how sensory stimuli paired with foot shock endow those formerly neutral stimuli with the ability now to potentiate startle for a very long time.

D. Role of Glutamate Receptors in the Amygdala in Fear-Potentiated Startle

Several studies have shown that high-frequency stimulation of amygdala afferents can result in a long-term potentiation (LTP) of neurotransmission at amygdala synapses. The mechanisms that underlie LTP may be similar to those engaged by fear conditioning (e.g., Rogan, Staubli, and LeDoux, 1997). Because the induction but not the expression of LTP most often involves NMDA receptors, we wondered whether amygdala NMDA receptors might also play a special role in fear learning. In fact, we found that local infusion into the basolateral nucleus of the amygdala, which has the highest density of NMDA receptors in the amygdala, of the NMDA antagonist AP5 blocked the acquisition but not the expression of fear-potentiated startle using either a visual (Miserendino, Sananes, Melia, and Davis, 1990), auditory (Campeau, Miserendino, and Davis, 1992), or olfactory (Walker, Paschall, and Davis, 2005) cues as conditioned fear stimuli. Importantly, the same doses did not disrupt the ability of conditioned fear stimuli to potentiate startle when infused prior to testing. Because the amygdala is essential for the expression of fear-potentiated startle (Campeau and Davis, 1995a; Hitchcock and Davis, 1987; Kim, Campeau, Falls, and Davis, 1993; Sananes and Davis, 1992; Walker and Davis, 1997b), these findings indicate that the effects of NMDA receptor blockade on fear learning cannot be attributed to a general disruption of amygdala activity or to a more specific disruption of the ability of rats to process the CS. These findings also indicate that the effects on learning cannot be attributed to anxiolytic influences, insofar as such influences should also disrupt fear-potentiated startle when NMDA receptor antagonists are infused prior to testing.

Although the inability of pretest infusions to disrupt fear-potentiated startle indicates that the effects of pretraining infusions cannot be attributed to a failure to process the CS, it could still be argued that AP5-induced learning impairments are attributable to a disruption of processing of the foot shock. Although these infusions did not alter the degree to which rats reacted to foot shock, as argued earlier, this may simply be a measure of a brainstem, spinal cord reflex, not directly related to fear conditioning. However, Gewirtz and Davis (1997) reported that intra-amygdala AP5 infusions blocked second-order
fear conditioning—a procedure in which a previously trained CS substitutes for shock as the aversive reinforcing stimulus. In this study, rats received pairings of an auditory stimulus (i.e., first-order stimulus) and foot shock. On other days, the same rats were given second-order conditioning trials, in which a light (i.e., the second-order stimulus) was paired, not with shock, but with the fear-eliciting first-order auditory stimulus. Prior to these second-order conditioning trials, rats received intra-amygdala infusions of either artificial cerebrospinal fluid (ACSF) or D,L-AP5. When subsequently tested, both groups showed fear-potentiated startle to the auditory stimulus. However, rats that had received AP5 did not show fear-potentiated startle to the light. Because AP5 was only given prior to light–tone pairings, the ability of AP5 to block fear learning could not be attributed to analgesic actions or to a disruption of neural transmission in pathways that convey foot-shock information to the amygdala. Furthermore, in the same rats where AP5 blocked second-order fear conditioning using the noise as the reinforcement, AP5 did not disrupt fear-potentiated startle to the first-order auditory CS. These data strongly suggest that AP5 disrupted the acquisition of fear by preventing the association between light and noise, rather than by preventing amygdala activation by the noise stimulus that was used as the reinforcement in second-order conditioning.

More recently it has been found that local infusion of AP5 antagonists into the amygdala block the expression of several other measures of conditioned fear, including freezing. However, this appears to be due to actions of AP5 on a particular subtype of the NMDA receptor, the NR2A subtype, because infusion of ifenprodil, another NMDA antagonist, which acts at the NR2B and not the NR2A subtype, blocked acquisition of conditioned freezing without having any effect on its expression (Rodrigues, Schafe, and LeDoux, 2001).

E. Involvement of AMPA Receptors in the Basolateral and Central Nucleus of the Amygdala in Fear Learning

Because the basolateral complex is a primary site of sensory convergence within the amygdala (see earlier), we wondered whether this subdivision might play a more prominent role in fear acquisition as compared to the central nucleus of the amygdala. To our surprise, pretraining infusions of NBQX into either area significantly disrupted fear learning, suggesting that both areas play a role in conditioning (Walker and Davis, 2000). Although it is difficult to rule out completely the possibility that infusions into the central nucleus disrupted fear learning by diffusing to the basolateral amygdala, we believe this is unlikely. In an earlier study using the same dose (3 μg/side), infusion volume (0.3 μl), infusion rate (0.1 μl/min), and stereotaxic coordinates, we were able to demonstrate differential effects of infusions into the basolateral versus central.
nucleus infusions on light-enhanced startle (Walker and Davis, 1997b) — an anxiety paradigm in which sustained exposure to bright light elevates startle amplitude (Walker and Davis, 1997a). In that experiment, NBQX infusions into the basolateral amygdala but not the central nucleus of the amygdala disrupted light-enhanced startle. Also, basolateral but not central nucleus AP5 infusions were able to disrupt fear learning (Fanselow, Kim, Yipp, and De Oca, 1994), presumably because NMDA receptors are more highly concentrated within the basolateral as compared to the central nucleus of the amygdala (Monaghan and Cotman, 1985). Our results and those of others (cf. Samson and Pare, 2005) are consistent with idea that both areas participate in fear learning and recent evidence that long-term potentiation can occur in the central nucleus of the amygdala (Samson and Pare, 2005).

**XI. INTRACELLULAR EVENTS INVOLVED IN FEAR-POTENTIATED STARTLE**

**A. Broad-Based Survey of Gene Changes in the Amygdala Following Fear Conditioning**

Many years ago we found an increase in the immediate early gene, *c-fos*, in the amygdala in the presence of a conditioned fear stimulus (Campeau, Hayward, Hope, Rosen, Nestler, and Davis, 1991), an effect that has been replicated many times, looking at both *c-fos* message and c-Fos protein. Interestingly, *c-fos* most consistently is seen in the medial nucleus of the amygdala during fear or stress (cf. Campeau, Falls, Cullinan, Helmreich, Davis, and Watson, 1997), and this may explain why inactivation of this part of the amygdala is so effective in blocking the expression of fear-potentiated startle. Recently, we began a much wider-based, *in situ* hybridization analysis of gene expression in the brain associated with the acquisition of fear-potentiated startle. We examined 21 genes known to be involved in neural plasticity based on their induction with kainic acid–induced seizures (Ressler, Paschall, Zhao, and Davis, 2002). We found a substantial number of these genes were transcriptionally regulated during consolidation of fear conditioning in the amygdala as well as in several other brain areas (Table 12-2). These mRNA changes occurred only when the conditioned and unconditioned stimuli were paired and not when unpaired or when the unconditioned stimulus was presented alone. These results suggest fear memory consolidation occurs within a broad neural circuit that includes, but is not limited to, the amygdala. It is associated with early and late changes in gene expression of a variety of transcription factors, cytoskeletal proteins, adhesion molecules, and receptor stabilization molecules, which together may contribute to the neural plasticity underlying long-term memory in mammals.
### TABLE 12-2  Gene Changes in the Amygdala and Extra-Amygdala Areas After Fear Conditioning

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Product</th>
<th>KA-Inducible Areas</th>
<th>Changes in Fear Conditioning</th>
<th>Peak Time of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 fos</td>
<td>Transcription factor</td>
<td>hipp, amyg, striatum, ctx</td>
<td>+++</td>
<td>0–60 min</td>
</tr>
<tr>
<td>2 zif268/EGR1</td>
<td>Transcription factor</td>
<td>hipp, amyg, striatum, ctx</td>
<td>+++</td>
<td>0–60 min</td>
</tr>
<tr>
<td>3 jun</td>
<td>Transcription factor</td>
<td>hipp, amyg, ctx</td>
<td>++</td>
<td>0–60 min</td>
</tr>
<tr>
<td>4 Neurofilament-L</td>
<td>Cytoskeletal protein</td>
<td>dentate, amyg, pir ctx</td>
<td>+++</td>
<td>0–60 min</td>
</tr>
<tr>
<td>5 Gephyrin</td>
<td>GlyR/GABAR Anchor</td>
<td>Decrease-hipp, amyg, pir ctx</td>
<td>++</td>
<td>30–120 min</td>
</tr>
<tr>
<td>6 RC3/Neurogranin</td>
<td>2nd msgr modulation</td>
<td>Decrease-hipp, amyg, pir ctx</td>
<td>+++</td>
<td>30–240 min</td>
</tr>
<tr>
<td>7 Nurr1</td>
<td>Transcription factor</td>
<td>hpp, amyg, pir ctx</td>
<td>+++</td>
<td>1–2 hr</td>
</tr>
<tr>
<td>8 16C8</td>
<td>Protease inhibitor</td>
<td>dentate</td>
<td>++</td>
<td>2–4 hr</td>
</tr>
<tr>
<td>9 α-actinin</td>
<td>NMDAR/GluR anchor</td>
<td>hpp</td>
<td>++</td>
<td>2–4 hr</td>
</tr>
<tr>
<td>10 n-cadherin</td>
<td>ECM/cell adhesion</td>
<td>hpp</td>
<td>+</td>
<td>2–4 hr</td>
</tr>
<tr>
<td>11 ler5/RM5</td>
<td>Transcription factor</td>
<td>dentate, amyg</td>
<td>+/-</td>
<td>1–4 hr</td>
</tr>
<tr>
<td>12 Tenascin</td>
<td>ECM/cell adhesion</td>
<td>cortex</td>
<td>+/-</td>
<td>1–4 hr</td>
</tr>
<tr>
<td>13 VGF</td>
<td>Neuropeptide</td>
<td>hpp</td>
<td>+/-</td>
<td>1–4 hr</td>
</tr>
<tr>
<td>14 EGR2/Krox20</td>
<td>Transcription factor</td>
<td>dentate</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>15 CREM</td>
<td>Transcription factor</td>
<td>hpp</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>16 EGR4</td>
<td>Transcription factor</td>
<td>hpp, pir ctx</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>17 AAR-21</td>
<td>Signal transduction</td>
<td>dentate, pir ctx</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>18 Rheb2</td>
<td>Signal transduction</td>
<td>dentate, pir ctx</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>19 PAI-2</td>
<td>Phosphatase inhibitor</td>
<td>dentate</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>20 ab-crystallin</td>
<td>Chaperone protein</td>
<td>dentate, ctx</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>21 CRF/CRH</td>
<td>Neuropeptide</td>
<td>amyg, hypoth</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
1. Gephyrin

Although several genes were up-regulated in the amygdala following fear conditioning, mRNA that codes for a protein called gephyrin, involved in the clustering of GABA and glycine receptors, was down-regulated (Ressler, Paschall, Zhao, and Davis, 2002). We also found this to be true when we measured the protein, as well as seeing a decrease in the surface expression of GABA-A receptors in the basolateral amygdala after fear conditioning, as evidenced by decreased binding of H3-flunitrazepam (Chhatwal, Myers, Ressler, and Davis, 2005). Because a decrease in the level of this clustering protein would be expected to decrease GABA transmission, this suggests that fear conditioning leads to a period of increased excitability in the amygdala for several hours. This is interesting because it is not possible to establish long-term potentiation in amygdala brain slices unless GABA antagonists are added. Although this seems unphysiological, these results with gephyrin suggest that fear conditioning down-regulates GABA-A in the amygdala, perhaps to allow long-term potentiation to take place, which may be important for consolidation of long term memory.

2. Brain-Derived Neurotropic Factor (BDNF)

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophins, a family of structurally related proteins first described for their role in promoting neuronal survival and differentiation during development. Since its original discovery, a large number of studies have demonstrated that BDNF plays a diverse role in regulating neuronal structure and function in both the developing and adult central nervous system, as well as long-term potentiation and learning and memory in hippocampally dependent tasks (cf. Rattiner, Davis, and Ressler, 2005). We found that BDNF mRNA was elevated in the basolateral amygdala 2 hours following fear conditioning (Rattiner, Davis, French, and Ressler, 2004). Furthermore, levels of TrkB receptor immunostaining appeared to decline in the amygdala, while levels of phosphorylated Trk receptors increased following fear conditioning, suggesting activation and modification of the receptor triggered by BDNF binding. Dominant-negative inhibition of TrkB within the amygdala impaired fear-potentiated startle without disrupting baseline amygdala function. These results strongly suggest a requirement for TrkB signaling in the acquisition and consolidation of fear memory.

Until recently, mechanisms of neural plasticity within the amygdala have focused primarily on NMDA receptor-mediated events (Rodrigues, Schafe, and LeDoux, 2001; Walker and Davis, 2000). There is now increasing evidence that BDNF/TrkB-dependent mechanisms of neural plasticity act in parallel with NMDA-dependent mechanisms in many neuronal cell types. Recent work has shown that PI3 kinase (PI3K) is a critical intracellular media-
tor required for synaptic plasticity during fear conditioning (Lin, Yeh, Lu, Leu, Chang, and Gean, 2001). It is quite likely that activation of TrkB is a critical step in PI3K-mediated signaling within the amygdala because TrkB has been shown to be a powerful regulator of this kinase. In our studies we have found significant but not complete blockade of fear learning following disruption of TrkB signaling, suggesting that other intracellular cascades likely act in parallel with those activated by BDNF to mediate long-term fear conditioning. Future studies will examine the differential roles of NMDA- versus TrkB-dependent plasticity events within the amygdala as well as the interactions between the different intracellular pathways activated by these receptors.

3. c-AMP Response Element Binding (CREB)

Local infusion of MAP kinase inhibitors into the amygdala blocked acquisition but not expression of conditioned fear using freezing to a tone (Schafe, Atkins, Swank, Bauer, Sweatt, and LeDoux, 2000; Schafe, Nadel, Sullivan, Harris, and LeDoux, 1999), and we found similar results with fear-potentiated startle (Lu, Shi, Tang, and Davis, 2000). Because each of these kinases can phosphorylate the transcription factor, c-AMP response element binding protein (CREB) and because CREB is both downstream and upstream from BDNF, we wondered whether we could establish a role for CREB in the amygdala in fear conditioning. After finding too much toxicity with local infusion of CREB antisense, we used viral vector gene transfer to up-regulate CREB to see if it would facilitate fear conditioning using suboptimal parameters (massed, as opposed to spaced, training trials). We found a dramatic increase in the magnitude of fear-potentiated startle associated with increased CREB protein during training, but not testing, in the basolateral amygdala following local infusion of herpes simplex virus (HSV) CREB in the amygdala (Josselyn, Shi, Carlezon, Jr., Neve, Nestler, and Davis, 2001). This only occurred with massed and not spaced training, consistent with earlier work in Drosophila (Yin, Del Vecchio, Zhou, and Tully, 1995) and CREB mutant mice (Kogan, Frankland, Blendy, Coblenz, Marowitz, Schutz, and Silva, 1997). There was no effect when lights and shocks were not paired or when spaced training was given with weak versus strong shocks or on shock reactivity during training and no effect on short-term memory. These data represented the first instance of a gain of function in a mammal following an increase in CREB, localized to a specific brain region, and is considered by some to be the best single piece of evidence implicating CREB in mammalian memory and a role for CREB in the amygdala in fear conditioning (Nguyen, 2001). We have now replicated this using a totally different paradigm, namely social defeat in hamsters (Jasnow, Shi, Israel, Davis, and Huhman, 2005), which is an amygdala-dependent form of long-term fear conditioning in this species.
Neural Systems Involved in Fear and Anxiety

Thalamus

Hippocampus

Basolateral Amygdala

Cortex

Orbital Frontal Cortex

Hippocampus

Dorsal & Ventral Striatum

Central N. Amygdala and/or Lateral BNST

Choice behavior

Memory of emotional events?

Memory consolidation of emotional events, spatial learning

Instrumental approach or avoidance behavior

Autonomic, somatic signs of fear. Attention to significant stimuli

Autonomic, somatic signs of anxiety

FIGURE 12-9 Schematic diagram of the outputs of the basolateral nucleus of the amygdala to various target structures and possible functions of these connections.

B. Role of the Bed Nucleus of the Stria Terminalis in Anxiety

The BLA projects to a variety of brain areas that are involved in fear and anxiety (Fig. 12-9). Two structures are of particular interest — the central nuclei of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST). As we have seen, the CeA is critical for the expression and probably the acquisition of conditioned fear. Recall, however, that lesions of the BNST did not block fear-potentiated startle or conditioned freezing. However, the lateral BNST and CeA are anatomically, neurochemically, cytoarchitectonically, and embryologically related (c.f., Alheid, de Olmos, and Beltramino, 1995) and that the BNST has the same downstream projections as the CeA (Fig. 12-10). Hence, we wondered how it might be involved in fear and anxiety.

1. CRH-Enhanced Startle

Infusions of corticotrophin-releasing hormone (CRH) into the lateral cerebral ventricle markedly increase the amplitude of the acoustic startle response in rats, and this effect was blocked by the anxiolytic chlordiazepoxide (Swerdlow, Geyer, Vale, and Koob, 1986). CRH-enhanced startle did not occur with intrathecal infusion and was not disrupted by lesions of the paraventricular nucleus of the hypothalamus (Liang, Melia, Campeau, Falls, Miserendino, and Davis, 1992), indicating mediation by CRH receptors in the brain that did not involve activation of the hypothalamic–pituitary–adrenal axis. Lee and Davis (1997a, 1997b) found that excitotoxic lesions of the BNST but not of the septum, hippocampus, BLA, or CeA completely blocked CRH-enhanced
startle, as did intra-BNST infusions of the CRH antagonist αhCRH. Infusions of CRH directly into the BNST increased startle amplitude at doses much lower than those that were required with i.c.v. administration (80 versus 1,000 ng). Neither BNST lesions nor intra-BNST αhCRH infusions disrupted fear-potentiated startle. Moreover, local infusion of CRH into the CeA failed to increase startle amplitude (Liang, Melia, Campeau, Falls, Miserendino, and Davis, 1992), and infusion of αhCRH there failed to block CRH-enhanced startle.

3. Light-Enhanced Startle

Walker and Davis (1997a) described a new animal model of anxiety termed light-enhanced startle, in which startle amplitude is increased when rats are exposed to bright light for 20 minutes. Like CRH-enhanced startle, light-enhanced startle was dependent on the BNST and not on the CeA (Walker and Davis, 1997b). Thus, local infusion of the AMPA receptor antagonist NBQX into the BNST but not the CeA blocked light-enhanced startle. The amygdala was still involved, however, because local infusion of NBQX into the BLA did block light-enhanced startle, probably because it is visual information transmitted to the BNST through the BLA. Thus, as with the CRH
experiments described earlier, these experiments demonstrated a double disso-
ciation between the roles of the BNST and the CeA in startle increases
produced by fear-inducing or anxiogenic stimuli.

4. Long-Term Sensitization of the Acoustic Startle Response by
Repeated Foot Shock

To examine whether the inability of BNST lesions to block fear-potentiat­ed
startle was related to the strength of conditioning, we used a procedure in
which acquisition of fear-potentiated startle can be measured by giving a few
training and test trials each day (Gewirtz, McNish, and Davis, 1998; Kim and
Davis, 1993). Even at early time points, when fear-potentiated startle was rela-
tively weak, sham- and BNST-lesioned rats showed comparable levels of fear-
potentiated startle. Unexpectedly, BNST lesions did influence one aspect of
performance. In shocked but not nonshocked control rats, baseline startle
amplitude (i.e., startle amplitude to the 10 noise bursts delivered at the begin-
ning of each test session) grew steadily over the course of training. The increase
did not appear to reflect contextual fear conditioning, but seemed instead to
reflect a long-term sensitization to startle stimuli produced by repeated foot-
shock administration and was absent in BNST-lesioned rats.

XII. WHAT DOES THE BNST DO? A PROVISIONAL
HYPOTHESIS BASED ON RESULTS FROM FEAR-
CONDITIONING AND ACOUSTIC STARTLE STUDIES

Fear-potentiated startle to a specific cue is a highly predictable situation that
involves prior conditioning and uses a rather short cue that reliably predicts an
aversive event. Fear-potentiated startle develops very rapidly, once the light
comes on, and dissipates very quickly, once the light goes off (Davis, Schlesinger,
and Sorenson, 1989). In contrast, light-enhanced startle is a situation where
the animal is exposed to a potentially dangerous situation that is less predict­
able, does not depend on any obvious conditioning, and involves a long period
of anticipation that something bad might happen. To try to explain why
manipulations of the CeA affect fear-potentiated startle and not light-enhanced
startle and why manipulations of the BNST affect light-enhanced startle and
not fear-potentiated startle, we suggested two alternatives. One hypothesis was
that the CeA mediates conditioned fear responses, whereas the BNST mediates
unconditioned fear responses. Support for this idea came from the finding that
startle that was increased in the presence of the smell of fox feces, presumably
an unconditioned fear stimulus, was blocked by inactivation of the BNST but
not the amygdala (Fendt, Endres, and Apfelbach, 2003). More recently,
however, it was reported that post-training lesions of the BNST blocked the
expression of context conditioning measured with freezing (Sullivan, Apergis, Bush, Johnson, Hou, and Ledoux, 2004), which clearly is a conditioned response to a context previously paired with shock, a result not consistent with the idea that the BNST is only involved in unconditioned fear. Our second hypothesis was that maybe the CeA mediates fear reactions activated by relatively short stimuli in highly predictable situations, whereas the BNST mediates fear responses to relatively long cues under conditions where the perceived danger is not highly predictable and requires a sustained state of defensive preparedness. We now believe this second alternative is the right conclusion.

In the light-enhanced-startle paradigm we found the light had to be on for at least 5 min to see maximal light-enhanced startle. At shorter intervals (i.e., 60 sec) the excitatory effect was weak, and at very brief intervals (i.e., 3.2 sec) the effect of light is often inhibitory (Davis, Schiesinger, and Sorenson, 1989). When the light is turned off, after a 20-min on-time, startle does not abruptly return to baseline but remains elevated for sometime thereafter (Walker and Davis, unpublished observations; de Jongh, Groenink, van der Gugten, and Olivier, 2002). Thus, light-enhanced startle requires a long-duration stimulus, and the effect of this stimulus far outlasts the period when the light is actually on. It also is an inherently unpredictable situation where the animal may feel "at risk" without knowing exactly when something might happen and how bad it might be. In fact, in humans we find that startle is increased in the dark (Grillon, Pellowski, Merikangas, and Davis, 1997) and that this effect is much larger in patients with posttraumatic stress disorder (Grillon, Morgan, Davis, and Southwick, 1998). When we asked these patients how they felt when the light went out, they often reported they felt like they were back in their bunker, anticipating a mortar attack but not knowing when this would happen.

CRH-enhanced startle may be similarly characterized. CRH-enhanced startle appears to be a slow-onset (20 min) and slow-offset effect (several hours), at least with i.c.v. administration (Lee and Davis, 1997a; Liang, Melia, Miserendino, Falls, Campeau, and Davis, 1992). It is not clear whether this protracted time course and slow decay of CRH-enhanced startle reflects response characteristics of the BNST itself, the time required for CRH to occupy and then dissociate from CRH receptors, or emergent properties of the neural circuitry within which the BNST is embedded. For example, Koob (1999) suggested that CRH-responsive neurons in the BNST and elsewhere, once activated by emotional stressors, excite brainstem noradrenergic nuclei, which then feed back to CRH-responsive neurons to stimulate further CRH release.

The effect on startle of repeated foot shock fits the pattern also. In Gewirtz, McNish, and Davis (1998), the effect developed gradually over many days and persisted for at least 24 hours (i.e., the interval between the final shock on the preceding training day and the baseline test on the following day).
TABLE 12-3 Role of the BNST in Anxiety and Stress

- Cue-induced drug craving
- Opiate withdrawal
- Social defeat
- "Learned helplessness"
- Context conditioning
- Predator odors

Overall, then, the data presently available argue for the existence of two phenomenologically and anatomically dissociable response systems, each capable of mediating increases in the amplitude of the acoustic startle response (Fig. 12-10). One, which includes the CeA as an integral component, can be characterized as a rapid response system that mediates short-term responses to specific threat cues (i.e., stimulus-specific fear responses). The other, which includes as an integral component the BNST, can be characterized as a sluggish response system that, once activated, continues to influence behavior long after the initiating stimulus has been terminated. We refer to the first, a stimulus-specific, short-lasting type of response, as “fear” and the second, a more sustained type of response, as “anxiety.” Moreover, these two different systems show perfect additivity, consistent with independent, parallel systems that elevate startle (Walker and Davis, 2002). Finally, many other laboratories are finding that the BNST plays a more general role in stress, depression, and anxiety, using many different experimental paradigms, including drug craving and withdrawal (Table 12-3, cf Walker, Toufexis, and Davis, 2003).

XIII. EXTINCTION OF FEAR-POTENTIATED STARTLE

If, following fear-potentiated startle to a visual stimulus, the light is presented over and over again without shock, there will be a significant decrease in the magnitude of fear-potentiated startle as a direct function of the number of presentations of the light in the absence of shock (Walker, Ressler, Lu, and Davis, 2002). This procedure is known as extinction training, and the theoretical process that accounts for this decrease in conditioned fear is known as extinction. Behavioral observations indicate that extinction is a form of learning in its own right, rather than an “unlearning” or forgetting of previous learning (for a review see Myers and Davis, 2002). We found that local infusion in the BLA of the NMDA antagonist AP5 completely blocked the development of extinction when animals were tested the next day drug free (Falls, Miserendino, and Davis, 1992). This impairment could not be attributed to an effect on NMDA receptors outside the amygdala, to damage to the amygdala, or to an impairment of sensory transmission during extinction training, and it has
been confirmed in several laboratories. Blocking NMDA receptors after extinction training also blocks extinction, suggesting that NMDA receptors are important for the consolidation of extinction (Santini, Muller, and Quirk, 2001).

In light of these findings, the question arose as to whether it would be possible to enhance extinction by enhancing the functioning of the NMDA receptor. It is known that a compound called D-cycloserine (DCS) binds to the NMDA receptor and makes it work better. Thus, we predicted that giving DCS prior to extinction training would enhance extinction. D-Cycloserine given either systemically or directly into the amygdala prior to extinction training dose-dependently enhanced extinction in rats exposed to lights in the absence of shock but not in control rats that did not receive extinction training when testing occurred 24 hr later in the absence of the drug (Walker, Ressler, Lu, and Davis, 2002), an effect now replicated with freezing to a tone (Ledgerwood, Richardson, and Cranney, 2003). This group also found that DCS could still facilitate extinction when given up to about 3 hr after extinction training, a finding consistent with the idea that DCS facilitates consolidation of extinction.

**XIV. FROM BENCH TO BEDSIDE**

Because treatments for PTSD and other anxiety disorders typically involve a process similar to extinction, we tested whether DCS would enhance exposure-based psychotherapy in people suffering from an inordinate fear of heights in a double-blind placebo controlled study. The exposure therapy used a virtual-reality situation developed by Barbara Rothbaum and colleagues in which patients rode in a virtual glass elevator to progressively higher floors (Ressler, Rothbaum, Tannenbaum, Anderson, Graap, Zimand, Hodges, and Davis, 2004). This situation is very frightening to patients just entering treatment, but it becomes considerably more tolerable with increasing exposure to the virtual environment, typically over six to eight sessions. Thirty patients were rated for their initial fear of heights and divided into three groups that had comparable levels of fear as well as being similar on other variables, such as age and sex, and then they received only two exposure sessions, purposely suboptimal, to detect improvement. Single doses of placebo or D-cycloserine (50 or 500 mg) were taken 2 hrs prior to each of the two sessions of virtual-reality exposure therapy. Exposure therapy combined with D-cycloserine resulted in significantly larger reductions of acrophobia symptoms on all main outcome measures than the same amount of exposure in combination with placebo. Compared to subjects receiving the placebo, subjects receiving DCS had significantly more improvement within the virtual environment both 1
week and 3 months after treatment. They also showed significantly greater decreases in posttreatment skin conductance fluctuations and greater improvement on general measures of real-world acrophobia symptoms and number of self-exposures to real-world heights. Because of these promising results, DCS is now being tested in combination with psychotherapy all over the world for all the major anxiety disorders.

**XV. SUMMARY**

By studying how a simple reflex can be augmented when elicited in the presence of a fearful stimulus (fear-potentiated startle), we and others have been able to delineate a good deal about the neural circuitry involved in conditioned fear. This involves visual or auditory as well as shock pathways that project via the thalamus and perirhinal or insular cortex to the basolateral nucleus of the amygdala (Bla). The Bla projects to the central (CeA) and medial (MeA) nuclei of the amygdala, which project indirectly to a particular part of the acoustic startle pathway in the brainstem. NMDA and BDNF receptors, as well as various intracellular cascades, in the amygdala are critical for fear learning, which is then mediated by glutamate acting in the CeA and perhaps substance P acting in the MeA. Less predictable stimuli, such as a long-duration bright light and a fearful context, activate the Bla, which projects to the bed nucleus of the stria terminalis (BNST), which projects to the startle pathway, much as the CeA does. The anxiogenic peptide corticotrophin-releasing hormone increases startle by acting directly in the BNST. Based on various differences between CeA- and BNST-mediated behaviors, we have suggested that CeA-mediated behaviors represent stimulus-specific fear, whereas BNST-mediated behaviors are more akin to anxiety. NMDA receptors are also involved in the extinction of conditioned fear, and both extinction in rats and exposure-based psychotherapy in humans are facilitated by an NMDA partial agonist called d-cycloserine.

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Neural Systems Involved in Fear and Anxiety


