PERGAMON

Neuroscience and Biobehavioral Reviews 23 (1999) 743–760

NEUROSCIENCE AND BIOBEHAVIORAL REVIEWS

www.elsevier.com/locate/neubiorev

The neuroanatomical and neurochemical basis of conditioned fear

M. Fendt[a,*], M.S. Fanselow[b]

[a]*Tierphysiologie, Universität Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany*
[b]*Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90095, USA*

Received 16 August 1998; received in revised form 1 March 1999; accepted 14 March 1999

Abstract

After a few pairings of a threatening stimulus with a formerly neutral cue, animals and humans will experience a state of conditioned fear when only the cue is present. Conditioned fear provides a critical survival-related function in the face of threat by activating a range of protective behaviors. The present review summarizes and compares the results of different laboratories investigating the neuroanatomical and neurochemical basis of conditioned fear, focusing primarily on the behavioral models of freezing and fear-potentiated startle in rats. On the basis of these studies, we describe the pathways mediating and modulating fear. We identify several key unanswered questions and discuss possible implications for the understanding of human anxiety disorders. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Conditioned fear; Fear-potentiated startle; Conditioned stimulus; Unconditioned stimulus; Periaqueductal gray; Amygdala; Freezing

1. Introduction

Acute fear can be one of the most potent emotional experiences of our lifetime. The strength of this subjective experience may be because fear serves a function that is critical to the survival of higher vertebrates. It can be thought of as activation of a defensive behavioral system [1] that protects animals or humans against potentially dangerous environmental threats. For a small vertebrate such as a rat, an example of such an environmental threat would be predation. These threats may be innately recognized or learned [2,3]. For example, in the presence of a cat or a stimulus that predicts potential injury, a rat will become completely motionless and freeze, no movements except those associated with respiration are observable [4–6]. Furthermore, the rat shows a fear-potentiated startle response [7–9], analgesia [10], a host of autonomic changes [11,12] and increased release of several hormones [13]. In humans, these responses are correlated with a subjective state of fear [14–17]. The brain and body are dedicated to fast and effective defense to increase the chances of survival. Therefore, we use the term "fear" to refer to the activation of the defensive behavioral system that gives rise to this constellation of reactions to threatening stimuli.

There are three major reasons why scientists investigate the neuronal basis of fear. First, they use fear-modulated behaviors as models to understand how emotions influence behavior. Second, the investigation of the neuroanatomical and neurochemical basis of fear and anxiety is a prerequisite to develop strategies to treat and cure anxiety disorders. Anxiety disorders, such as specific phobias (agoraphobia, social phobia, etc.), panic disorder, post-traumatic stress disorder and generalized anxiety disorder are among the most common psychopathologies in the industrial states. Third, fearful experiences are rapidly learned about and long remembered. Hence, fear-conditioning has become an excellent model for trying to unravel the processes and mechanisms underlying learning and memory.

The development of several reliable behavioral tasks for investigating fear has led to major developments in our understanding of the neuronal basis of fear and anxiety in just the last decade. These behavioral tasks fall into two general classes: learned and unlearned. Tests of unlearned fear rely on stimuli that naturally provoke fear even when the animal has had no prior experience with the stimulus. The most frequently used stimuli in these tasks are natural predators (e.g. [18]) and exposure to a novel place (especially one that is brightly lighted [19] or elevated [20]). Approaches using learned fear examine conditioned behaviors provoked by stimuli that have become associated with something aversive, usually an electric footshock. These Pavlovian fear stimuli provoke many of the same behaviors that innate fear stimuli do. For example, rats freeze to both cats and conditioned stimuli associated with shock. To

* Corresponding author. Tel.: + 49-7071-297-5347; fax: + 49-7071-292-618.

E-mail address: markus.fendt@uni-tuebingen.de (M. Fendt)

0149-7634/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0149-7634(99)00016-0

measure the conditioned fear, a number of specific responses can be easily quantified such as fear-potentiated startle [7,8], freezing [3,21], tachycardia [22], conditioned defensive burying [23] and ultrasonic vocalization [24–26]. Alternatively, conditioned fear can be measured as a disruption of ongoing behaviors (e.g. conditioned suppression [27,28] and conflict tests [29,30]).

Validity for this approach to fear is obtained when a variety of stimuli that present clear threats to the subject generate a consistent set of behaviors that are tailored to protect against the threat. Additionally, these perceptual-motor organizations should have a common neuronal basis that overlaps considerably with the neural systems that mediate human fear and anxiety. Furthermore, the potency of drugs that modulate human fear and anxiety should correlate with their effectiveness in altering the behavior in these animal models.

The present review will primarily compare two specific responses to learned fear, freezing and fear-potentiated startle because these are most clearly identified with specific neural mechanisms that mediate between environmental stimulus and behavioral response. We go on to describe a hypothetical neuronal circuit which characterizes conditioned fear and helps organize existing knowledge about several conditioned defensive behaviors. Finally, we indicate what we feel to be some of the most critical open questions remaining for the analysis of fear.

2. The fear-conditioning procedure

Fear-conditioning is a form of Pavlovian conditioning where a subject is trained to associate a neutral stimulus (e.g. a 10 s presentation of light) with an aversive, unconditioned stimulus (US), such as an electric footshock. After such pairings, the light alone predicts the occurrence of the shock and acts as a conditioned stimulus (CS), eliciting a state of fear. Tones, lights, odors and tactile stimuli have been used as CS in fear-conditioning experiments. These stimuli range from a few seconds to a few minutes in duration and because of this brevity are called discrete CS. However, the subject also has fear responses conditioned to the setting in which the discrete CS and shock US was presented. Such stimuli, which are less temporally restricted and are made up of many separate features, are referred to as contextual stimuli. The fear of contextual and discrete CS can be acquired as rapidly as a single trial.

When examining the neural circuitry mediating fear learning, there are three different time-points at which experimental manipulations can be made. Manipulations during the training procedure affect the acquisition of conditioned fear, while manipulations during the testing procedure affect the expression of conditioned fear. If consolidation of a fear memory is to be targeted, the manipulation is made after acquisition, but before testing. If a brain structure were lesioned, the time-point when the lesion was carried out can help us to make a statement about the influence of this lesion on the acquisition or on the expression of conditioned fear. If this brain structure were only involved in the acquisition of conditioned fear, only pre-training, and not post-training lesions would affect the measure of fear. On the contrary, if this brain structure was only involved in the expression of conditioned fear, both the pre- and post-training lesions should affect conditioned fear. Manipulations that affect consolidation are usually temporally graded such that the greatest effect occurs when the manipulation is carried out immediately after training. Obviously, reversible treatments provide the most powerful tools for separating acquisition, consolidation and expression processes.

2.1. Fear-potentiated startle

A startle response is elicited by a sudden acoustic, visual or tactile stimuli and is found in every mammal studied so far [31]. A typical startle response is composed of a fast, sequential muscle contraction, with the most prominent reaction around the face, neck and shoulders [15,31]. Possible functions of the startle response are to reduce the latency of a flight reaction [32] and/or a protection from a predator's attacks from behind by contraction of the dorsal muscles [33]. The electromyographically measured latency of the startle response in rats is only 5–10 ms [34,35], indicating a relatively short neuronal startle pathway with only a few central synapses. The elementary startle pathway was initially described by Davis and co-workers [36], and further detailed by Lingenhöhl and Friauf [37,38] and Lee and co-workers [39]. It includes the cochlear root neurons, the giant neurons of the caudal pontine nucleus of the reticular formation (PnC) and spinal motorneurons. Yeomans and Frankland [33] suggested a further parallel pathway additionally including the ventrolateral pons and spinal interneurons. In the last decade, the startle response became a valuable model for investigating behavioral modulations such as habituation [40,41], sensitization [42,43], prepulse inhibition [44,45] and Pavlovian conditioning [7,8]. Furthermore, appetitive emotions weaken the startle response [17,46,47], while aversive emotions such as fear or anxiety enhance the startle response [7,8,13,17,41].

The fear-potentiated startle paradigm was initially described by Brown, Kalish and Farber [7]. Rats are given several pairings of a light CS and footshock. After this procedure, the mean amplitude of the acoustic startle response to a loud noise is usually 50–100% higher in the presence of the light CS than to the noise alone [8,48]. The difference between these two trial types (light-noise and noise alone) represents the fear-potentiation of the startle response and acts as a measure of fear. Fear-potentiated startle is very sensitive to drugs that are known to modulate the state of fear: norepinephrine antagonists [49], benzodiazepine agonists [50], dopamine antagonists [8], opioid agonists [51], 5-HT_{1A} agonists [52], 5-HT_3 antagonists

[53], corticotropin-releasing factor antagonists [54], cholecystokinin antagonists [55], neuropeptide Y agonists [56], NMDA-associated glycine receptor antagonists [57], NMDA antagonists [57] and ethanol [58,59] block or reduce the fear-potentiation of the startle response after systemic injections (reviewed in Refs. [8,60]). Most of these drugs were also tested in humans and had an anxiolytic effect [61–65].

Sensitization of the acoustic startle response is another approach used to investigate the effects of aversive stimuli on reflexes. Sensitization of the startle response is the immediate enhancement of startle amplitude after shock [42]. Initially this excitatory effect of footshock on startle was thought to be an unconditioned response to footshock [42]. Recent work indicates that sensitization reflects a rapid conditioning to the test environment [66,67]. Therefore, it is suggested that the mechanisms underlying the sensitization of the startle are largely identical to those that mediate fear-potentiated startle [68].

2.2. Freezing

Over a century ago, Darwin recognized that fear produces a profound suppression of activity in several species (see [69, p. 260]). Small [21] reported freezing as a characteristic fear response of rats and Griffith [5] reported that rats would show pronounced freezing in the presence of a cat. While freezing was reported to occur in aversive conditioning experiments using shock, it was initially considered a nuisance variable (e.g. [70]). Earlier studies of Pavlovian conditioned fear used measures such as bar press suppression that relied on what the rat was not doing (i.e. it had stopped eating [71,72]). Investigation of direct observational measures of freezing (e.g. [4,73]) and crouching [74] to shock associated cues began in earnest in the early 1970s, but these typically looked at reactions to contextual cues. Direct measures of freezing to discrete CS such as tones and lights began in Robert Bolles' laboratory in the late 1970s (e.g. [75,76]). Bouton and Bolles [77] showed that direct visual observation of freezing to tones paired with shock provided a measure that correlated highly with, but tended to be more sensitive than, other measures such as conditioned suppression. There tends to be no baseline freezing in control rats that have not received shock. There is reliable freezing with even a single brief (0.75 s) mild (0.5 mA) shock conditioning trial, and more robust training parameters can easily result in freezing levels near 100% (e.g. [78]). For rats, freezing is a highly selected response because movement makes the rat more detectable to predators and because predators are much more likely to attack moving than still prey. In other words, movement acts as a releasing stimulus for predatory attacks. This is probably why freezing is observed even in situations that afford the opportunity for other behaviors such as escape (see [79] for a review).

2.3. Other behavioral indices

There are certainly other behavioral manifestations of fear in aversive Pavlovian conditioning situations. Obviously, there are profound changes in autonomic function. Defecation covaries with other measures of fear [80] and blood pressure shows a reliable increase (e.g. [81–84]). While fear CS influence heart rate as well, these changes are much less consistent than the hypertensive effects of fear stimuli [84]. Both tachycardia (e.g. [22]) and bradycardia (e.g. [85,86]) have been reported. What determines the direction of the heart rate change is not clear at this time, but whether or not the rat is restrained [86,87], the type of conditioning control one uses [88] and the baseline heart rate (e.g. [85]) appear to contribute and possibly interact.

There are, of course, other responses that characterize the fear response. For example, rats show ultrasonic vocalizations (e.g. [24–26]) and a loss in pain sensitivity [89,90]. Such responses add to the validity that the fear state is related to a species typical survival function (e.g. [10,91,92]).

3. The role of the amygdala

It is now well established that the amygdala plays a pivotal role in fear. The initial hints of this were provided by Brown and Schaffer [93] who reported that large lesions of the temporal lobe tamed previously ferocious monkeys. Kluver and Bucy [94] characterized the rather widespread emotional disturbance caused by such brain damage and this psychopathology became known as the Kluver–Bucy syndrome. Weiskrantz [95] reported that many aspects of the Kluver–Bucy syndrome could be produced by damage restricted to the amygdala. Fuster and Uyeda [96] were the first to show that there were cells within the amygdala that selectively respond to a CS paired with shock. Subsequently, it has been confirmed that cells in both the central [97] and lateral nuclei [98] of the amygdala show short latency CS specific activity. The fact that these neurons in the amygdala will show increased responsiveness to stimuli after they were paired with shock indicates that the structure is sensitive to the convergence of the CS and US information. The dorsal subdivision of the lateral amygdala may be important for the processing of this convergence as it has cells that respond to both tones and footshock [99].

Stimulation of afferent pathways to the amygdala can lead to an enhanced responsiveness of cells in the amygdala; in other words, the amygdala shows long-term potentiation (LTP [100–103]). Using lateral amygdala slices, Huang and Kandel [102] showed that LTP depends on post-synaptic depolarization and calcium influx into the post-synaptic cell. As this LTP accludes paired-pulse facilitation, Maren and Fanselow suggested that the potentiation was expressed through a pre-synaptic mechanism [103]. Huang and Kandel [102] have subsequently confirmed this observation.

Glutamate receptors, particularly NMDA receptors, play a critical role in these responses [102–104]. Indeed, fear-conditioning itself can potentiate amygdala responses [105,106]. Together these electrophysiological data indicate that the amygdala has the potential to be a point where the CS and US converge to produce fear-conditioning. Therefore, in the next sections we examine the amygdala's contribution to two specific behavioral indices of conditioned fear, freezing and fear-potentiated startle.

3.1. The amygdala and fear-potentiated startle

The first studies investigating the neuroanatomical basis of fear-potentiated startle were carried out in the mid 1980s. A series of studies by Davis and colleagues showed that the pathway from the amygdala to the PnC is essential for the potentiation of the startle response by conditioned fear. First, they showed that fear-conditioning potentiates the startle response at the level of the PnC [107,108]. Second, lesions of the amygdala blocked fear-potentiated startle using a visual CS [109] or an auditory CS [110], while lesions of other nuclei (e.g. the cerebellum or the red nucleus, which are both known to be involved in Pavlovian conditioning of reflexive responses [111,112]) had no effect. Third, destruction of the direct pathway from the central nucleus of the amygdala to the PnC—the ventral amygdalofugal pathway [113]—blocked fear-potentiation of the startle response [114]. Thus, the amygdala is necessary to observe fear-potentiation of startle.

Activity in the amygdala is sufficient for potentiation of startle as electrical stimulation there increases the amplitude of the startle response [115–117]. Koch and colleagues showed a strong short-latency potentiation of the startle amplitude after injections of glutamate into the central nucleus of the amygdala [118], confirming that it was the activity of neurons intrinsic to the amygdala that potentiated the response. A longer latency increase of startle amplitude could be produced when selective metabotropic glutamate receptor agonists were applied to the amygdala [119].

3.1.1. Acquisition of fear-potentiated startle

To test the hypothesis that NMDA receptor-dependent LTP in the amygdala mediates fear-conditioning, Davis and colleagues microinjected NMDA receptor antagonists (AP-5 and AP-7) and pertussis toxin into the basolateral nucleus of the amygdala. These LTP-impairing treatments blocked acquisition, consistent with the suggestion that NMDA receptors in the basolateral nucleus of the amygdala are involved in the plasticity underlying fear-conditioning [120–122]. Interestingly, the elimination of fear-potentiated startle during extinction is an NMDA-dependent process, as well. Injections of AP-5, but not of the non-NMDA receptor antagonist CNQX into the basolateral amygdala blocked extinction of fear-potentiated startle [123].

Gewirtz and Davis [124] extended these results to second-order conditioning. This occurs when a previously conditioned CS (first-order CS) is paired with another CS (second-order CS). The first-order CS functions like a US, giving the second-order CS the ability to produce a conditioned response. Injections of AP-5 into the basolateral nucleus of the amygdala during the acquisition of second-order fear-conditioning blocks the acquisition of fear-potentiation to the second-order CS. Interestingly, the expression of fear-potentiation by the first-order CS was slightly increased during testing. One explanation of this finding is that AP-5 blocked the extinction of the first-order CS that normally occurs during second-order training.

A lesion study by Tischler and Davis [125] led to the initial hypothesis that the amygdala receives information about a visual CS via a pathway from the retina to the dorsal lateral geniculate nucleus to the visual cortex to the deep layers of the superior colliculus and down to the elementary startle pathway. Further extensive lesion studies by the Davis group [126–129] suggested that the basolateral and/or the lateral nucleus of the amygdala receives CS information from the perirhinal cortex. Auditory CS are mediated from the cochlea via different subnuclei of the auditory thalamus to the perirhinal cortex, while visual CS are mediated from the retina via the lateral geniculate body to the perirhinal cortex.

There are several routes by which information about shock can reach the amygdala, but it seems unlikely that any single one of these pathways is necessary and sufficient as a US pathway for conditioning. Fendt and colleagues [130] suggested that US information for fear-potentiated startle is carried from the spinal cord, through the nucleus paragigantocellularis and the locus coeruleus to the amygdala. This was based on the finding that the locus coeruleus is activated by footshock via the nucleus paragigantocellularis [131–133], which in turn projects to the amygdala. The locus coeruleus-amygdala pathway uses noradrenaline as a transmitter [134] and a reduction of noradrenaline release in the amygdala blocks the enhanced startle seen immediately after footshock [130]. However, the hypothesis that noradrenaline release in the amygdala mediates the reinforcing aspects of the US was contradicted by the finding that a blockade of amygdaloid β-adrenergic receptors has no effect on the acquisition of fear-potentiated startle [122].

The central nucleus of the amygdala also receives nociceptive information via a projection from the nucleus parabrachialis [135,136]. The transmitters of this projection are mainly neuropeptides but also noradrenaline [137]. While this pathway may make a contribution to conditioning the fact that, at least in rat, there do not appear to be projections from the central nucleus to the lateral nucleus suggests that this pathway cannot support the CS–US convergence found in the lateral nucleus [138].

Based on anatomical tracing and electrolytic lesions experiments, Shi and Davis [139,140] recently suggested that two parallel pathways can provide the amygdala with nociceptive input. Footshocks information is conveyed from the spinal cord to the basolateral nucleus of the amygdala

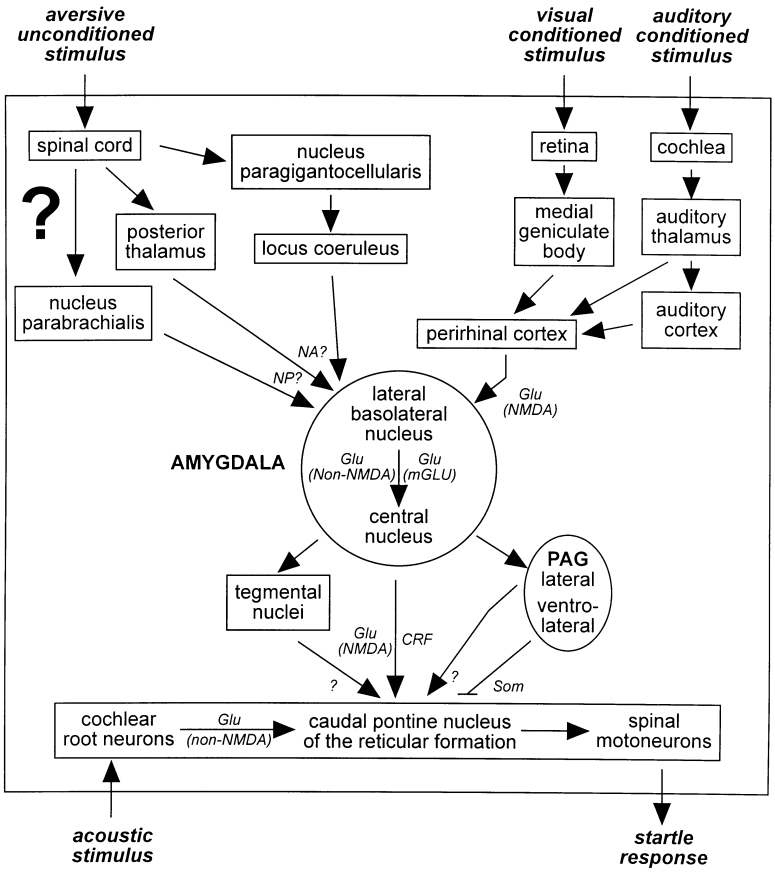

Fig. 1. Hypothetical circuit mediating fear-potentiated startle. Abbreviations: CRF, corticotropin-releasing factor; Glu, glutamate; NA, noradrenaline; NP, neuropeptides; Som, somatostatin.

via a direct pathway that synapses in the posterior intralaminar nucleus. An additional indirect pathway includes synapses in the ventral posteriolateral thalamic nucleus, the posterior thalamic nucleus, the posterior intralaminar nucleus, the areas S1 and S2 and the caudal insular cortex. Acquisition of fear-potentiated startle was only blocked when both the direct and indirect pathways were lesioned, thus either one is sufficient to support conditioning. As combined lesions in these pathways affected acquisition but not expression of fear-potentiated startle they may indeed function as parallel US pathways. However, the pattern of data also leaves open the possibility that these pathways modulate memory storage within the amygdala.

3.1.2. Expression of fear-potentiated startle

Injections of the non-NMDA receptor antagonist CNQX [141] or NBQX [142] but not of the NMDA receptor antagonist AP-5 [120] into the central or the basolateral nucleus of the amygdala blocked the expression of fear-potentiated startle. This suggests that fear-potentiation is mediated by a projection from the lateral and/or basolateral nucleus of the amygdala to the central nucleus of the amygdala activating non-NMDA receptors. As intra-amygdaloid injections of the CCK_B receptor agonist pentagastrin [143,144] increased the baseline startle amplitude and systemic injections of CCK_B antagonists blocked the fear-potentiated startle [55], amygdaloid CCK_B receptors seem to be involved in the expression of fear-potentiated startle, as well.

The central nucleus of the amygdala is the origin of a direct pathway to the elementary startle pathway [113,118,145], mediating the expression of fear-potentiated startle [114]. Koch and Ebert [146] showed that the effect of amygdaloid stimulation on the activity of PnC neurons can be blocked by microiontophoretic applications of the NMDA receptor antagonist AP-5 into the PnC. These results and the fact that AP-5 microinjections into the PnC block fear-potentiated startle [147] suggested that the direct pathway from the central nucleus of the amygdala to the PnC mediates fear-potentiated startle, uses glutamate as a transmitter and acts via NMDA receptors. This hypothesis is supported by previous studies, showing that NMDA receptors in the PnC are involved in the up-modulation of the startle response, while the non-NMDA receptors are involved in the direct mediation of the startle response [148–150]. Anatomical experiments showed that the direct pathway from the central nucleus of the amygdala to the PnC uses the neuropeptide corticotropin-releasing factor (CRF) as a transmitter [145]. Microinjections of CRF receptor antagonists into the PnC block the expression of

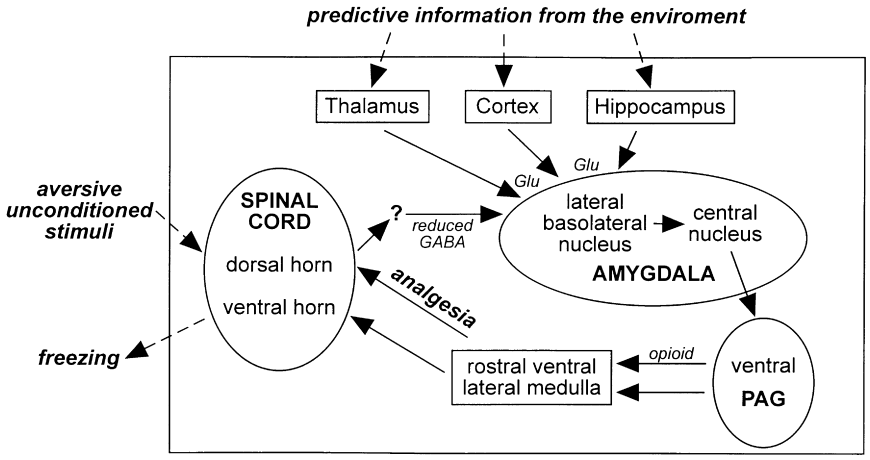

Fig. 2. Hypothetical circuit mediating conditional fear-induced freezing and opioid analgesia. Abbreviations: Glu, glutamate; GABA, γ-amino butyric acid.

fear-potentiated startle [145], while injections of CRF into the PnC increased the baseline startle response [151].

Conditioned inhibition of fear-potentiation of startle occurs when a second stimulus (e.g. a tone) signals that the CS (e.g. a light) will not be followed by the usual footshock during training [152]. Conditioned inhibition was not blocked by amygdaloid lesions suggesting that conditioned inhibitors acts through another brain structure [153].

3.1.3. Summary

The amygdala is a critical structure for the acquisition of fear-potentiated startle. Information about the CS and the US converge in the lateral, and perhaps basolateral amygdala (Fig. 1). A direct pathway from the central nucleus of the amygdala to the PnC uses CRF and glutamate as a transmitter and modulates the primary startle circuit to produce fear-potentiation.

3.2. The amygdala and freezing

The involvement of the amygdala in freezing to shock associated cues was first demonstrated by Blanchard and Blanchard [2], who found that large lesions of the amygdala abolished freezing to a context associated with shock. LeDoux and colleagues extended this earlier findings by showing that selective destruction of cells within the lateral amygdala block freezing to auditory CS [154]. This critical role of the amygdala has been confirmed for virtually all CRs to fear stimuli as blood pressure [154], heart rate [155], analgesia [156,157] and ultrasonic vocalizations [158] are blocked by amygdala lesions. Fig. 2 indicates a circuit responsible for the mediation of two of these fear CRs, freezing and opioid analgesia.

3.2.1. Acquisition of freezing

The basolateral complex of the amygdala, particularly the lateral and basolateral nuclei, appears to be critical for acquisition of freezing. Blockade of NMDA receptor activity [159–161] or enhanced GABAergic inhibition [162–164] within the amygdala during acquisition blocks the expression of freezing in the undrugged state. Consistent with the electrophysiological and fear-potentiated startle data reviewed before, the lateral and/or basolateral nuclei seem to be the important site of CS–US convergence and neural plasticity as AP-5 prevented acquisition when given to the basolateral complex but not when injected into the central nucleus [159]. Extinction of fear-induced freezing to both the discrete and contextual CS is also prevented by administering AP-5 to the basolateral complex [160].

Although fear-potentiated startle studies have typically used a light as a discrete CS, freezing experiments have typically used tone. Information about the discrete CS, provoking freezing, appears to arrive at the amygdala via direct thalamo-amygdala and also thalamo-cortico-amygdala projections [154]; either pathway appears to be sufficient for mediating a conditioned freezing response to tone. The primary pathway mediating freezing to a discrete CS appears to be the direct thalamo-amygdala pathway. The cortico-amygdala pathway may serve more complex fear-related information-processing, and also provide a redundant pathway capable of supporting simple conditioning as well [165–167]. The properties of freezing make it ideal for analyzing fear of more static, or contextual cues [78,168]. The hippocampal formation appears to play a critical role in providing the amygdala with information about contextual CS [86,103,169].

As with fear-potentiated startle, the nature of the pathway carrying US information is not clear, even though footshock is known to evoke responses in the lateral amygdala [99]. Currently, the best candidate is the spinothalamic tract, which carries somatosensory information about the shock US to the posterior intralaminar nucleus (PIN) of the thalamus. The PIN is immediately ventromedial to the areas of the medial geniculate that carry auditory CS information [170,171]. Tone footshock pairings result in altered tuning curves of cells in the medial geniculate [172] and electrical stimulation of the PIN can serve as an US for conditioned bradycardia in rabbits to auditory stimuli [170]. It is not

known if this specific pathway or some analogue supports conditioning in other CS modalities. Additionally, because lesions that damaged the PIN did not prevent acquisition of freezing to a tone paired with footshock, other US pathways must be sufficient. As was shown with fear-potentiated startle, the insular cortex may be the redundant pathway [140], but that has yet to be tested for freezing.

In a more general sense, GABA antagonism has been found to function as an US for Pavlovian fear-conditioning [173] and as mentioned before, GABAergic agonists block acquisition of conditioned fear. Transgenic mice with the B_3 subunit of the $GABA_A$ receptor deleted show an impairment in the acquisition of conditioned freezing [174]. Therefore, it seems possible that a reduction in tonic GABAergic inhibition at the amygdala acts as the ultimate effect of the US to promote conditioning.

There is a serious conceptual problem with any potential US pathway for fear-conditioning if fear-conditioning is to be linked to a mechanism of cellular plasticity like LTP. The LTP analogy suggests that a CS cannot initially activate cells that can produce fear, but it acquires the ability to do so because it is paired with a US that can effectively depolarize these cells. This would suggest that the US should be capable of generating the constellation of fear responses that the LTP is presumed to support. However, while CS paired with shock readily produce freezing as a conditioned response, the shock US itself has no ability to provoke freezing [80,168]. Future research will need to reconcile this discrepancy between behavior and cellular mechanism, but the neural basis of how the US fosters learning currently stands as the most open question in the acquisition of Pavlovian fear.

3.2.2. Expression of freezing

As with fear-potentiated startle, the amygdala is important for expression of conditioned fear-induced freezing. If a rat is trained with an intact amygdala, excitotoxic lesions of the structure abolish expression of freezing to both tone and contextual CS even when a substantial consolidation period is given between training and testing [175]. Amygdala application of lidocaine, muscimol and diazepam all block expression of freezing [156,162,163]. AP-5 blocks expression of freezing to both discrete [160] contextual stimuli [161], and this contrasts with the lack of effect of AP-5 on the expression of fear-potentiated startle to a fear-inducing tone [120] or light [122]. However, these effects of AP-5 are consistent with the finding that AP-5 also blocks evoked potentials in the lateral and basolateral nuclei in response to electrical stimulation of the pathways carrying information about contextual [103] and discrete CS to the amygdala [104].

As with fear-potentiated startle, the central nucleus acts as an output pathway to brain structures that generate freezing. However, for freezing these projections from the central nucleus terminate in the midbrain rather than the brain stem.

3.3. Other models

The involvement of the amygdala in several other indices of Pavlovian fear appears to be consistent with the data from freezing and fear-potentiated startle. Kapp and colleagues [155] were the first to show that amygdala lesions blocked autonomic responses to Pavlovian fear stimuli—in this case it was conditioned bradycardia in rabbits. Iwata et al. extended this finding to arterial hypertension in the rat [154]. Conditioned fear-induced analgesia is also blocked by amygdala lesions [176].

In humans, damage to the amygdala precludes fear-conditioning as assessed by changes in skin conductance [177,178]. While the emotional component fear of conditioning was blocked in these patients, as long as the hippocampus was intact they remembered the events that happened during training. This indicates that the amygdala is specifically involved in learning the emotional aspects of the fear-conditioning experience. Other, non-emotional information is encoded in parallel by other brain systems.

The data reviewed above indicate four important points: (1) a large number of very different indices of conditioned fear are abolished by amygdala lesions; (2) this structure receives convergence of CS and US information; (3) pharmacological manipulations targeted at neural plasticity in this structure also abolish learning; and (4) evoked activity in this structure shows changes following Pavlovian fear-conditioning. When these are taken together, the inescapable conclusion is that the amygdala is a crucial structure for the learning of fear.

The central nucleus may be the end of the common pathway mediating fear as “fear state” and it appears that different efferents from the central nucleus mediate different fear responses. Central nucleus projections to the PnC mediate the fear-potentiation of startle. However, efferents to the lateral hypothalamus [179] and medulla, e.g. [180], mediate autonomic responses. Finally, projections to the periaqueductal gray (PAG) are critical for freezing and analgesia [81,179,181], but may be important for the expression of fear-potentiated startle, as well [48,181–183]. Indeed, second to the amygdala, the PAG may be the most critical area in the brain for fear and defensive behaviors [184,185].

4. The role of the periaqueductal gray

4.1. The periaqueductal gray and fear-potentiated startle

Cassella and Davis [186] first showed that the PAG is involved in the modulation of startle responding. They reported that electrolytic lesions of the dorsal PAG enhanced baseline amplitude, habituation and sensitization of the startle response. Although these lesions increased the sensitization of the startle response, no influences on the potentiation of startle by conditioned fear could be observed [186]. Some of Cassella’s and Davis’ data were supported

later by Borszcz et al. [187], showing that electrolytic lesions of the ventrolateral PAG enhance both short-term and long-term habituation. Chemical PAG lesions by Fendt and co-workers [184] totally blocked the sensitization of the startle response without affecting the baseline startle amplitude. Furthermore, anatomical data of this study showed a possible indirect pathway from the central nucleus of the amygdala via the lateral PAG to the PnC mediating the effects of aversive stimuli on the startle response. A follow-up study showed that PAG lesions prevent fear-potentiated startle [48], suggesting that the PAG is involved in the mediation of fear-potentiated startle too. In both lesion studies, mainly the lateral and the dorsal part of the PAG was lesioned.

Walker and Davis [188] chemically lesioned the dorsolateral PAG more rostrally than the lesions of Fendt and colleagues, and found that these lesions did not block fear-potentiated startle if the rats were trained with moderate footshock (0.6 mA). If strong footshocks (1.6 mA) were used, fear-potentiated startle was reliable only in lesioned rats but not in control rats. Furthermore, chemical stimulation of the dorsolateral PAG reduced fear-potentiated startle without affecting baseline startle amplitudes. The authors suggested that the dorsolateral PAG is activated by particularly aversive events and this activation may interfere with the expression of fear-potentiated startle.

These data suggest that different regions of the PAG differentially influence fear-potentiated startle. For example, weak chemical stimulation of the lateral PAG enhances fear-potentiated startle [182] and electrical stimulation of the same area increases the startle baseline amplitude [189], while chemical stimulation of the ventrolateral PAG attenuates the expression of fear-potentiated startle [182].

4.1.1. Expression of fear-potentiated startle

Fendt and colleagues [48] lesioned the PAG before and after the fear-conditioning training procedure. Both the pre- and post-training lesions prevented fear-potentiated startle, indicating that the PAG is certainly involved in the expression of fear-potentiated startle. However, these experiments do not rule out a potential role of the PAG in the acquisition of fear-potentiated startle. Further experiments are necessary to resolve this question.

4.1.2. Inhibition of fear-potentiated startle

Anatomical and electrophysiological experiments revealed a somatostatinergic projection from the ventrolateral PAG to the PnC, which may act to reduce the excitatory effects of glutamate on tone-evoked activity of the PnC [190]. Weak chemical stimulation of the ventrolateral PAG led to a decrease of fear-potentiated startle [181,182] and injections of somatostatin into the PnC dose-dependently reduced fear-potentiation of the startle response [190]. These results suggested that this somatostatinergic projection from the ventrolateral PAG to the PnC is involved in the inhibition of fear-potentiated startle.

Recent results suggest that inhibition of fear-potentiated startle by the ventrolateral PAG is not involved in conditioned inhibition of fear-potentiation of startle as chemical stimulation of the ventrolateral PAG decreased the fear-potentiated startle, but did not affect the conditioned inhibition of fear-potentiated startle [182]. In contrast, there are indications that the dorsal PAG is involved in the mediation of conditioned inhibition, as chemical stimulation of the dorsal PAG reduces conditioned inhibition of fear-potentiated startle [182].

4.1.3. Summary

A pathway from the central nucleus of the amygdala to the PnC via the lateral PAG is involved in the mediation of the effects of conditioned fear on the elementary startle circuit. Additionally, the ventrolateral PAG has a somatostatinergic projection to the elementary startle circuit, which is involved in the inhibition of fear-potentiated startle.

4.2. The periaqueductal gray and freezing

As stated earlier, the PAG is absolutely critical for freezing. Liebmann et al. [191] discovered the PAG's involvement in this response when they found that rats with large lesions of the PAG did not freeze following an extended series of strong shocks. Lesions of the PAG eliminate freezing of rats not only to conditioned fear stimuli but to cats as well [92]. These lesions attenuate conditioned freezing when made either before or after training [192]. The ventrolateral PAG seems to be the region critical for freezing. First, lesions of the PAG that completely spare the tissue ventral and ventrolateral to the aqueduct do not reduce freezing [193]. Furthermore, lesions of the dorsal raphe that spare the surrounding ventral PAG also fail to reduce freezing [194]. Carrive and colleagues [185] examined Fos immunoreactivity in rats following exposure to a context previously paired with shock. They found that these rats both froze and showed the greatest number of Fos stained nuclei in the ventrolateral column of the PAG compared to the control.

As with fear-potentiated startle, dorsolateral PAG lesions have a modulatory effect on freezing. Dorsolateral PAG lesions made before, but not after training, will enhance the level of freezing observed on testing [192,193]. However, this enhancing effect is confined to training parameters that show paradoxically reduced freezing because of very dense shock schedules.

Within the PAG, expression of the unconditioned response and the conditioned response to shock can be doubly dissociated [181]. Lesions of the dorsolateral PAG reduce the unconditioned burst of activity produced by the shock, but do not reduce the conditioned freezing. Ventrolateral regions have the opposite effect; they reduce conditioned freezing but do not affect the unconditioned activity

burst. This dissociation further illustrates the profound separation of the CR and the UR in Pavlovian fear-conditioning.

4.3. Other models

While direct stimulation of the PAG can have pronounced autonomic effects [195], it has been repeatedly demonstrated that the autonomic reactions to conditioned fear stimuli do not depend on the PAG [81,179]. However, like freezing, fear-induced analgesia depends on the PAG as lesions of this structure block the reduction in pain sensitivity produced by conditional fear [81]. Within the PAG, freezing and analgesia are dissociable as injections of the opioid antagonist naltrexone into the ventral PAG block analgesia but not freezing [196]. This conditioned fear-induced analgesia is realized from projections from the PAG to the rostral ventromedial medulla [81]. Fig. 2 summarizes this information.

5. Other brain regions

Although the amygdala and PAG play a central role in the acquisition and expression of fear-related behavior, certainly several other brain regions play an important role as well. In the ensuing paragraphs, we will discuss the two brain regions that have been shown to play a role in fear-potentiated startle and freezing, the tegmental area and the hippocampal formation, respectively.

5.1. Tegmental nuclei

The tegmental nucei play a role in fear-potentiated startle, but this area is yet to be examined for freezing response. Sensitization of the startle response after application of footshock is blocked by microinjections of substance P antagonists into the PnC [197]. This indicates that the laterodorsal tegmental nucleus is involved in the potentiation of the startle response by fear, as the laterodorsal tegmental nucleus is the only brain structure providing substance P-ergic input to the PnC [198]. Electrolytic lesions of the midbrain tegmental area (including the lateral tegmental nucleus) blocked fear-potentiation of the startle response [114].

Frankland and Yeomans [199] made chemical lesion of the rostrolateral midbrain, a brain area including the lateral tegmental nucleus, and showed that these lesions also block fear-potentiated startle. They suggested that a further parallel pathway from the amygdala via the rostrolateral midbrain (the lateral tegmental nucleus?) to the brainstem is involved in the mediation of fear-potentiated startle. Anatomical tracing studies showed that the amygdalofugal pathway (including the direct pathway from the amygdala to the PnC) cross the midbrain tegmental nuclei but there is also a projection from the central nucleus of the amygdala terminating in this area [113,183].

The ventral tegmental area (VTA) plays a role in fear-potentiated startle [189]. Chemical lesions of the VTA blocked the expression of fear-potentiated startle. Electrical stimulation of the VTA enhanced the baseline startle amplitude and increased the fear-potentiation of the startle response, while microinjections of the $D_{2/3}$ receptor antagonist quinpirole into the VTA totally blocked the fear-potentiated startle [190].

Injections of CCK-8S, a CCK receptor agonist, into the PnC increase the baseline startle amplitude [200], suggesting that an excitatory CCK-ergic projection to the PnC is involved in the expression of fear-potentiated startle. The VTA, the central nucleus of the amygdala and the PAG show a high density of CCK containing neurons [201] and project to the PnC, so any or all of these projections may use CCK as a transmitter.

5.2. Hippocampus

As might be expected from its role in spatial [202] and/or configural [203] learning, the hippocampus plays a disproportionate role in the fear acquired in the situation where fear-conditioning occurred. When tones were paired with shock, lesions of the hippocampus blocked freezing to the contextual cues associated with shock, but the same rats froze normally to the tone [204,205]. Lesions of the hippocampus made shortly after training produce a severe retrograde amnesia for contextual fear [169,206]. If the lesions are made prior to conditioning, anterograde amnesia is also observed, although it seems to be less pronounced than retrograde amnesia [206–208]. Retrograde amnesia for conditioned freezing to contextual cues is time-limited, as the interval between training and lesion increases the retrograde amnesia decreases [169,206,208]. The effects of hippocampal lesions on freezing to contextual cues are remarkably selective, in a way that accords well with the human amnesic syndrome [209]. Some forms of memory are drastically impaired (context conditioning), while the others are spared (conditioned freezing to auditory cues) and the type of memory that is lost shows a temporal gradient for retrograde amnesia [210].

McNish et al. [211] reported that while lesions of the hippocampus disrupt freezing to contextual cues, they do not affect the fear-potentiated startle to the same contextual cues. Unfortunately, a flaw in this study makes it premature to conclude that the hippocampus plays a different role in these two measures of contextual fear. McNish et al. did not include an assessment of the effects of hippocampal lesions on baseline startle magnitude. As the hippocampal lesions have been reported to increase the baseline startle response [212], the effects of hippocampal lesions on fear-potentiated startle would be masked by any increases in baseline startle response. It should be noted that the specificity of the deficit in the contextual freezing described before, indicates that hippocampal lesions do not affect the rat's ability to freeze [207,209]. Given the very selective effects of hippocampal

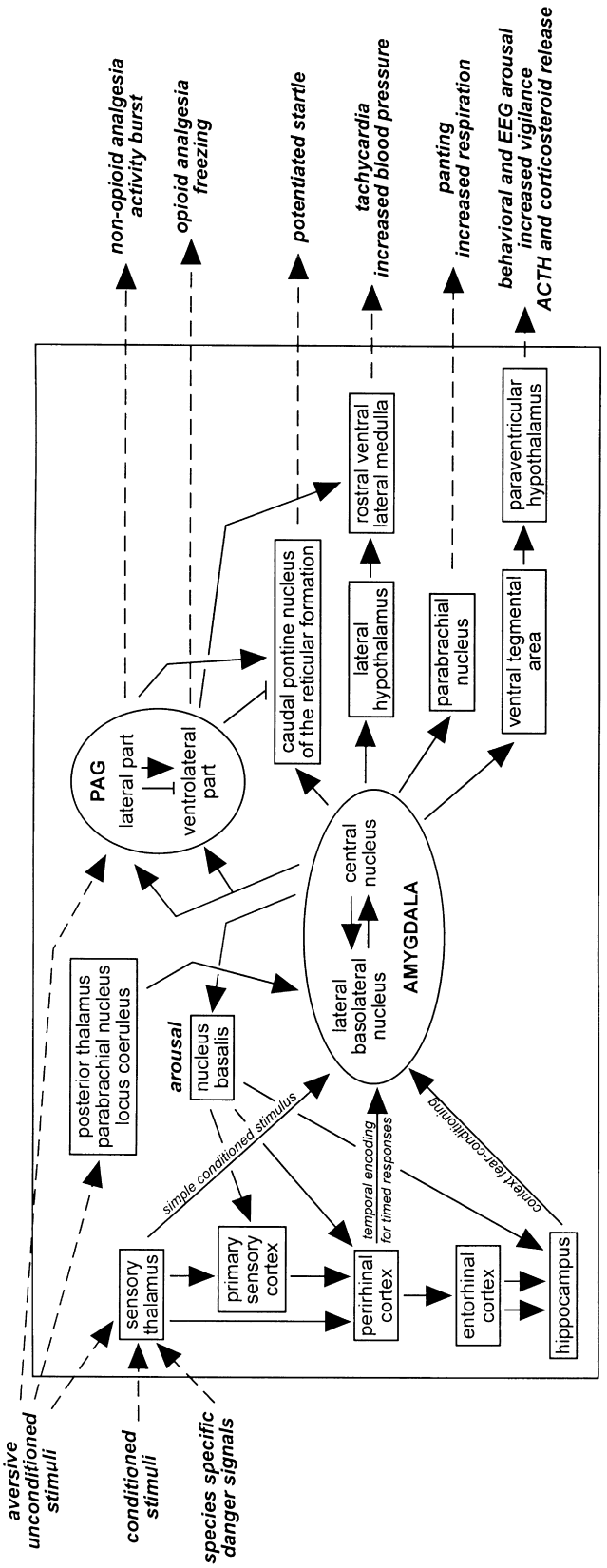

Fig. 3. Hypothetical circuit mediating the different aspects of conditioned fear. Abbreviations: ACTH, adrenocorticotrophic hormone; EEG, electro-encephalogram; PAG, periaqueductal gray.

lesions on fear and its more general role in memory, it seems likely that its function is to convey a configural or spatial memory of context to the amygdala, where it can be associated with shock [103]. This places the hippocampus on the input side of fear-conditioning.

6. Summary, a neural circuitry and open questions

The studies reviewed here suggest a certain neural circuitry and this is shown in Fig. 3. This circuit goes a long way in integrating and summarizing the extensive data on Pavlovian fear-conditioning.

As shown in the figure, the amygdala plays the central role in the acquisition and expression of fear to the conditioned stimulus [136,213–216]. The amygdala is the interface between the sensory system that carry information about the CS and US, and the different motor and autonomic systems that control the conditioned reactions. If any one structure is to be associated with the acquisition of conditioned fear, it is the lateral and/or basolateral amygdala. It seems likely that the cellular mechanism underlying this learning is NMDA-receptor dependent long-term potentiation (LTP) [106,217–219].

The amygdala receives information about the US from several sources that act in parallel. How they act to foster association formation is unknown. The problem arises because in fear-conditioning the conditioned and the unconditioned response are different; the US conditions responses it does not normally activate.

The CS pathway to the amygdala seems well characterized. As is shown in Figs. 2 and 3, the lateral and basolateral amygdala receive direct input from the thalamus as well as cortically processed input via the perirhinal cortex and hippocampal formation. What function do each of these pathways serve? As has been described earlier, for the hippocampus the case seems clear; information processed by the hippocampus normally functions to provide contextual information to the amygdala [169,205]. However, the available data does not provide any clear picture for a differential role of cortico- and thalamo-amygdala projections in excitatory fear-conditioning to discrete CS. The pathways seem to function somewhat redundantly as either route seems sufficient to support fear-conditioning on its own [126,167,220]. The sorts of discriminations, such as sound localization, considered to depend on the auditory cortex have yet to be tested with fear-conditioning [221–223]. After sufficient overtraining, fear-potentiated startle seems to peak at the point when the US is normally delivered [224]. Such temporal encoding is likely to require processing in cortical regions such as the perirhinal cortex [225].

There also seem to be pharmacological differences in the pathways that carry CS information to the amygdala. Projections from the hippocampal formation [103] and medial geniculate body [104,226] use both the NMDA and the AMPA receptors for normal synaptic transmission, as NMDA antagonists reduce evoked potentials in the amygdala produced by stimulation of these structures. On the contrary, AMPA and not NMDA antagonists [104,226] reduce amygdala responses to activity in auditory cortex. Expression of freezing to conditioned tone and contextual stimuli is reduced by NMDA antagonists applied to the amygdala of rats trained in the absence of drug [160,161], but expression of fear-potentiated startle to a tone CS is not affected by NMDA antagonists [120]. The most straightforward explanation of this pattern is that fear-potentiated startle depends on a cortico-amygdala glutamatergic pathway that requires only AMPA activity to drive action potentials. However, thalamo- and hippocampo-amygdala glutamatergic pathways that require both the NMDA and the AMPA currents for the generation of action potentials may drive freezing. Note that the Campeau et al. [120] study used a relatively large number of training trials and examined a response that is well timed [224]. The freezing study used few trials and a response that is not particularly well-timed [161]. Thus, the pattern of data is consistent with the idea that cortico-amygdala projections are particularly important for temporal encoding that is revealed when very discrete fear responses are observed in overtrained animals. Certainly, this hypothesis is in need of further analysis.

Given the schema presented here and the generally devastating effects of amygdala lesions on Pavlovian conditioned fear, one might expect that rats with amygdala lesions might never express fear-related behavior. While this seems to be the case under normal training conditions, recent data suggests that fear may be present when extensive overtraining is given. Kim and Davis [227] found that while rats given extensive overtraining completely lost fear (as measured by fear-potentiated startle) following amygdala lesions, fear could be reacquired by these overtrained rats. Reacquisition of fear-potentiated startle progressed to near normal levels. Using the freezing preparation, Maren [228] found a similar pattern. However, reacquisition was only complete in animals that had partial lesions of the basolateral nucleus. Animals with total basolateral lesions still showed a large, albeit incomplete, deficit despite pre-lesion overtraining. Maren went on to show that this same pattern was obtained when the lesions were made before training. Including the central nucleus in the lesion did not alter the pattern of behavior. Thus, pre-training while intact does not appear to be the critical variable in the survival of fear following amygdala lesions. Rather the two crucial factors are the amount of spared amygdala tissue and the amount of training. With the freezing measure there is significant acquisition in a single trial and freezing is asymptotic at about six trials. In animals with complete basolateral lesions, freezing was abolished even with 25 training trials and was still significantly impaired at 35 trials. Killcross and co-workers [229] gave far more extensive training in a complex discrimination task and found that while some components of the fear response reached near normal levels, others were still dramatically impaired. Thus, without an

amygdala fear responses never appear completely normal, although very extensive overtraining allows some expression of the standard fear measures (fear-potentiated startle and freezing). It remains to be demonstrated what brain structures allow this residual fear-related behavior.

Within the present framework, the amygdala is playing the role of sensory-motor interface for fear. The simplest translation of this theory would suggest that when the amygdala is functional, all the fear responses it is essential for should occur in concert. Data on the ontogeny of fear calls this most parsimonious version into question [85,229,231]. Fear to tones, lights and contexts first develops at different ages with tone appearing first and context appearing last [85,231]. This is true for at least three measures of fear (freezing, fear-potentiated startle and heart rate changes). However, these different measures of fear also appear at different ages; with freezing appearing first and fear-potentiated startle appearing last [85,230]. Thus, a 23-day old rat can freeze and show fear-potentiated startle to a tone associated with a shock, but only freezing and not fear-potentiated startle, is observed to a light associated with shock [85]. This ontological pattern suggests that the sensory-motor organization within the amygdala and its afferent structures is quite complex. Note that this pattern cannot be due simply to maturation of structures afferent to the amygdala (i.e. sensory information) as there is an age at which rats will respond with freezing to a light CS, but not show fear-potentiated startle to the same stimulus. However, the pattern cannot be simply maturation of response pathways either. Rats will show fear-potentiated startle to a tone before they can show it to a light.

Obviously, there are different pathways mediating the expression of conditioned fear. The PAG seems to be involved in the expression of several measures of conditioned fear (e.g. analgesia, freezing and fear-potentiated startle). Whether the different fear responses have common or separated processing in the several regions of the PAG, should be a question of further study. Another important aim of future investigation should be elucidation of neurochemical differences in the different pathways from the amygdala to the other brain structures mediating different fear responses. For example, the parabrachial nucleus mediates changes in respiration, the lateral hypothalamus and parts of the medulla oblongata mediate cardiovascular responses. The bed nucleus of the stria terminalis mediates stress reactions and the ventral tegmental area and the paraventricular hypothalamus seem to be involved in the modulation of arousal and vigilance by conditioned fear (reviewed in Refs. [136,213–216]). Once the amygdala recognizes that the situation predicts danger, it generates the constellation of fear responses through multiple parallel and sometimes redundant channels. These pathways may mediate slightly different aspects of fear, allowing fine-tuning of the ultimate behavioral response to fear-provoking stimuli under a variety of external and internal conditions. This implies that different parallel pathways make the “fear system” more plastic, and thus more responsive to variable external demands. A ripe area for future research is in the coordination of these various response components of fear into integrated and functional defensive behavior [92,181]. Additionally, the neurochemical separation of these various pathways may allow the development of new drugs capable of differentiating between the several behavioral and autonomic problems that humans with anxiety disorders suffer.

In humans suffering from anxiety disorders, this system is functioning so effectively that fear is disproportionate to the actual threat predicted by the situation. Therefore, the investigation of the neuroanatomy and the neurochemistry of extinction and inhibition of conditioned fear could be a key to new strategies in the treatment of anxiety disorders. As would be expected, analysis of the neural mechanisms that inhibit fear lags far behind that of the mechanisms that produce fear. Specific drugs could enhance the extinction of the associations that produce fear or increase the inhibition of fear-related behavior and autonomic changes of patients at critical moments.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 307/C2 and Fe 483/1-1) to M.F. and National Science Foundation (US) grant # IBN-9723295 to M.S.F. M.F. specially thanks Dr. Michael Koch for helpful discussions during the work on the manuscript.

References

[1] Fanselow MS. The midbrain periaqueductal gray as a coordinator of action in response to fear and anxiety. In: Depaulis A, Bandler R, editors. The midbrain periaqueductal gray matter, New York: Plenum Press, 1991. pp. 151.
[2] Blanchard DC, Blanchard RJ. Innate and conditioned reactions to threat in rats with amygdaloid lesions. J Comp Physiol Psychol 1972;81:281–290.
[3] Blanchard RJ, Blanchard DC. Defensive reactions in the albino rat. Learn Motiv 1971;2:351–362.
[4] Bolles RC, Collier AC. Effect of predictive cues on freezing in rats. Anim Learn Behav 1976;4:6–8.
[5] Griffith CR. The behavior of white rats in the presence of cats. Psychobiology 1920;2:19–28.
[6] Morris BJ. Neuronal localisation of neuropeptide Y gene expression in the rat brain. J Comp Neurol 1989;290:358–368.
[7] Brown JS, Kalish HI, Farber IE. Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. J Exp Psychol 1951;41:317–328.
[8] Davis M, Falls WA, Campeau S, Kim M. Fear-potentiated startle: a neural and pharmacological analysis. Behav Brain Res 1993;58:175–198.
[9] Leaton RN, Borszcz GS. Potentiated startle: its relation to freezing and shock intensity in rats. J Exp Psychol: Animal Behav Proc 1985;11:421–428.
[10] Bolles RC, Fanselow MS. A perceptual-defensive-recuperative model of fear and pain. Behav Brain Sci 1980;3:291–301.
[11] Black AH, de Toledo L. The relationship among classically conditioned responses: heart rate and skeletal behavior. In: Black AH,

Prokasy WF, editors. Classical conditioning. II. Current theory and research, New York: Appleton (Century/Crofts), 1972 pp. 290–311.
[12] LeDoux JE. Brain mechanisms of emotion and emotional learning. Curr Op Neurobiol 1992;2:191–197.
[13] Davis M, Hitchcock JM, Rosen JB. Neural mechanism of fear conditioning measured with the acoustic startle reflex. In: Madden IV J, editor. Neurobiology of learning, emotion and affect, New York: Raven Press, 1991 pp. 67–95.
[14] Bradley MM, Lang PJ, Cuthbert BN. Emotion novelty and the startle reflex: habituation in humans. Behav Neurosci 1993; 107:970–980.
[15] Brown P. Physiology of startle phenomena. In: Fahn S, Hallett M, Lüders HO, Marsden CD, editors. Negative motor phenomena, Philadelphia: Lippincott-Raven Publishers, 1995 pp. 273–287.
[16] Howard R, Ford R. From the jumping Frenchmen of Maine to post-traumatic stress disorder: the startle response in neuropsychiatry. Psychol Med 1992;22:695–707.
[17] Lang PJ, Bradley MM, Cuthbert BN. Emotion attention and the startle reflex. Psychol Rev 1990;97:377–395.
[18] Blanchard RJ, Blanchard DC, Agullana R, Weiss SM. Twenty-two kHz alarm cries to presentation of a predator by laboratory rats living in a visible burrow system. Physiol Behav 1991;50:967–972.
[19] Montgomery KC, Monkman JA. The relation between fear and exploratory behavior. J Comp Physiol Psychol 1955;48:132–136.
[20] Graeff FG, Viana MB, Tomaz C. The elevated T maze a new experimental model of anxiety and memory: effect of diazepam. Brazilian J Med Biol Res 1993;26:67–70.
[21] Small W. Notes on the psychic development of the white rat. Am J Psychol 1899;11:80–100.
[22] LeDoux JE, Sakaguchi A, Reis DJ. Subcortical efferent projections of the geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. J Neurosci 1984;4:683–698.
[23] Treit D, Pinel JP, Fibiger HC. Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. Pharmacol Biochem Behav 1981;15:619–626.
[24] Borszcz GS. Pavlovian conditional vocalizations of the rat: A model system for analyzing the fear of pain. Behav Neurosci 1995;109:648–662.
[25] Kaltwasser MT. Acoustic startle induced ultrasonic vocalization in the rat: a novel animal model of anxiety. Behav Brain Res 1991;43:133–137.
[26] Miczek KA, Weerts EM, Vivian JA, Barros HM. Aggression anxiety and vocalizations in animals: $GABA_A$ and 5-HT anxiolytics. Psychopharmacology 1995;121:38–56.
[27] English HB. Three cases of the “conditioned fear responses”. J Abn Social Psychol 1929;24:221–225.
[28] Millenson JR, Leslie J. The conditioned emotional response (CER) as a baseline for the study of anti-anxiety drugs. Neuropharmacology 1974;13:1–9.
[29] Dooley DJ, Klamt I. Differential profile of the CCK_B receptor antagonist CI-988 and diazepam in the four-plate test. Psychopharmacology 1993;112:452–454.
[30] Geller I, Seifter J. The effects of memprobamate barbiturates d-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1960;1:482–492.
[31] Landis C, Hunt WA. The startle pattern. New York: Farrar and Rinehart, 1939.
[32] Pilz PKD, Schnitzler HU. Habituation and sensitization of the acoustic startle response in rats: amplitude threshold and latency measures. Neurobiol Learn Memory 1996;66:67–79.
[33] Yeomans JS, Frankland PW. The acoustic startle reflex: neurons and connections. Brain Res Rev 1996;21:301–314.
[34] Caeser M, Ostwald J, Pilz PKD. Startle response measured in muscles innervated by facial and trigeminal nerves show common modulation. Behav Neurosci 1989;103:1075–1081.
[35] Cassella JV, Harty TP, Davis M. Fear conditioning prepulse inhibition and drug modulation of a short latency startle response measured electromyographically from neck muscles in the rat. Physiol Behav 1986;36:1187–1191.
[36] Davis M, Gendelman DS, Tischler MD, Gendelman PM. A primary acoustic startle circuit: lesion and stimulation studies. J Neurosci 1982;2:791–805.
[37] Lingenhöhl K, Friauf E. Giant neurons in the caudal pontine reticular formation receive short latency acoustic input: an intracellular recording and HRP-study in the rat. J Comp Neurol 1992;325:473–492.
[38] Lingenhöhl K, Friauf E. Giant neurons in the rat reticular formation: a sensorimotor interface in the elementary acoustic startle circuit . J Neurosci 1994;14:1176–1194.
[39] Lee Y, Lopez DE, Meloni EG, Davis M. A primary acoustic startle pathway: obligatory role of cochlear root neurons and the nucleus reticularis pontis caudalis. J Neurosci 1996;16:3775–3789.
[40] Moyer KE. Startle response: habituation over trials and days and sex and strain differences. J Comp Physiol Psychol 1963;56:863–865.
[41] Plappert CF, Pilz PKD, Schnitzler HU. Acoustic startle response and habituation in freezing and nonfreezing rats. Behav Neurosci 1993;107:981–987.
[42] Davis M. Sensitization of the acoustic startle reflex by footshock. Behav Neurosci 1989;103:495–503.
[43] Davis M, Cedarbaum JM, Aghajanian GK, Gendelman DS. Effects of clonidine on habituation and sensitization of acoustic startle in normal decerebrate and locus coeruleus lesioned rats. Psychopharmacology 1977;51:243–253.
[44] Hoffman HS, Searle JL. Acoustic variables in the modification of startle reaction in the rat. J Comp Physiol Psychol 1965;60:53–58.
[45] Swerdlow NR, Caine SB, Braff DL, Geyer MA. The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. J Psychopharmacol 1992;6:176–190.
[46] Koch M, Schmid A, Schnitzler HU. Pleasure-attenuation of startle is disrupted by lesions of the nucleus accumbens. NeuroReport 1996;7:1442–1446.
[47] Schmid A, Koch M, Schnitzler HU. Conditioned pleasure attenuates the startle response in rats. Neurobiol Learn Memory 1995;64:1–3.
[48] Fendt M, Koch M, Schnitzler HU. Lesions of the central gray block conditioned fear as measured with the potentiated startle paradigm. Behav Brain Res 1996;74:127–134.
[49] Davis M, Redmond DE, Baraban JM. Noradrenergic agonists and antagonists: effects on conditioned fear as measured by the potentiated startle paradigm. Psychopharmacology 1979;65:111–118.
[50] Davis M. Diazepam and flurazepam: effects on conditioned fear as measured with the potentiated startle paradigm. Psychopharmacology 1979;62:1–7.
[51] Davis M. Morphine and naloxone: effects on conditioned fear as measured with the potentiated startle paradigm. Eur J Pharmacol 1979;54:341–347.
[52] Kehne JH, Cassella JV, Davis M. Anxiolytic effects of buspirone and gepirone in the fear-potentiated startle paradigm. Psychopharmacology 1988;94:8–13.
[53] Nevins ME, Anthony EW. Antagonists at the serotonin-3 receptor can reduce the fear-potentiated startle response in the rat: Evidence for different types of anxiolytic activity. J Pharmacol Exp Ther 1994;268:248–268.
[54] Swerdlow NR, Britton KT, Koob GF. Potentiation of acoustic startle by corticotropin-releasing factor (CRF) and by fear are both reversed by α-helical CRF (9-41). Neuropsychopharmacology 1989;2:285–292.
[55] Josselyn SA, Frankland PW, Petrisano S, Bush DEA, Yeomans JS, Vaccarino FJ. The CCK_B antagonist L-365, 260 attenuates fear-potentiated startle. Peptides 1995;16:1313–1315.
[56] Broqua P, Wettstein JG, Rocher MN, Gauthier-Martin B, Junien JL. Behavioral effects of neuropeptide Y receptor agonists in the elevated plus-maze and fear-potentiated startle procedure. Behav Pharmacol 1995;6:215–222.

[57] Anthony EW, Nevins ME. Anxiolytic-like effects of *N*-methyl-D-aspartate-associated glycine receptor ligands in the rat potentiated startle test. Eur J Pharmacol 1993;250:317–324.
[58] Miller NE, Barry III H. Motivational effects of drugs: methods which illustrate some general problems in psychopharmacology. Psychopharmacologia 1960;1:169–199.
[59] Pohorecky LA, Cagan M, Brick J, Jaffe LS. The startle response in rats: effects of ethanol. Pharmacol Biochem Behav 1976;4: 311–316.
[60] Hijzen TH, Houtzager SWJ, Joordens RJE, Olivier B, Slangen JL. Predictive validity of the potentiated startle response as a behavioral model for anxiolytic drugs. Psychopharmacology 1995;118:150–154.
[61] Handley S. Future prospects for the pharmacological treatment of anxiety. CNS Drugs 1994;2:397–414.
[62] Harro J, Vasar E, Bradwejn J. CCK in animal and human research on anxiety. Trends Pharmacol Sci 1993;14:244–249.
[63] Olivier B, Molewijk E, van Oorschot R, van der Poel G, Zethof T, van der Heyden J, Mos J. New animal models of anxiety. Eur Neuropsychopharmacol 1994;4:93–102.
[64] Price LH, Goddard AW, Barr LC, Goodman WK. Pharmacological challenges in anxiety disorders. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress, New York: Raven Press, 1995 pp. 1311–1323.
[65] Rasmussen K. CCK schizophrenia and anxiety. Ann New York Acad Sci 1994;713:300–311.
[66] Pilz PKD. Sensitization of the acoustic startle response in rats is sensitive to a change of the environment. In: Elsner N, Schnitzler H-U, editors. Brain and evolution, Stuttgart: Georg Thieme, 1996. pp. 223.
[67] Richardson R, Elsayed H. Shock sensitization of startle in rats: the role of contextual conditioning. Behav Neurosci 1998;112:1136–1141.
[68] Koch M, Schnitzler HU. The acoustic startle response in rats - circuits mediating evocation inhibition and potentiation. Behav Brain Res 1997;89:35–49.
[69] Darwin C. The expression of the emotions an man and animals. Chicago: University of Chicago Press, 1960.
[70] Miller NE. An experimental study of acquired drives. Psychol Bull 1941;38:534–535.
[71] Annau Z, Kamin LJ. The conditioned emotional response as a function of intensity of the US. J Comp Physiol Psychol 1961;54:428–432.
[72] Estes WK, Skinner BF. Some quantitative properties of anxiety. J Exp Psychol 1941;29:390–400.
[73] Bolles RC, Riley AL. Freezing as an avoidance response: another look at the operant-respondent distinction. Learn Mem 1973;4:268–275.
[74] Blanchard RJ, Blanchard DC. Passive and active reactions to fear-eliciting stimuli. J Comp Physiol Psychol 1969;68:129–135.
[75] Collier AC. Preference for shock signals as a function of the temporal accuracy of the signals. Learn Mem 1977;8:159–170.
[76] Fanselow MS, Bolles RC. Naloxone and shock-eliciting freezing in the rat. J Comp Physiol Psychol 1979;93:736–744.
[77] Bouton ME, Bolles RC. Conditioned fear assessed by freezing and by the suppression of three different baselines. Anim Learn Behav 1980;8:429–434.
[78] Fanselow MS. Conditioned and unconditioned components of post-shock freezing. Pav J Biol Sci 1980;15:177–182.
[79] Fanselow MS, Lester LS. A functional behavioristic approach to aversively motivated behavior: predatory imminence as a determinant of the topography of defensive behavior. In: Bolles RC, Beecher MD, editors. Evolution and learning, New York: Hillsdale, 1988. pp. 185.
[80] Fanselow MS. Conditioned fear-induced opiate analgesia: a competing motivational state theory of stress analgesia. Ann New York Acad Sci 1986;467:40–54.
[81] Helmstetter FJ, Tershner SA. Lesions of the periaqueductal gray and rostral ventromedial medulla disrupt antinociceptive but not cardiovascular aversive conditional responses. J Neurosci 1994;14:7099–7108.
[82] LeDoux JE, Sakaguchi A, Reis DJ. Alpha-methyl-DOPA dissociates hypertension cardiovascular reactivity and emotional behavior in spontaneously hypertensive rats. Brain Res 1983;259:69–76.
[83] LeDoux JE, Sakaguchi A, Reis DJ. Strain differences in fear between spontaneously hypertensive and normotensive rats. Brain Res 1983;277:137–143.
[84] Pappas BA, DiCara LV, Miller NE. Acute sympathectomy by 6-hydroxydopamine in the adult rat: effects on cardiovascular conditioning and fear retention. J Comp Physiol Psychol 1972;79:230–236.
[85] Hunt PS, Campbell BA. Developmental dissociation of the components of conditioned fear. In: Bouton ME, Fanselow MS, editors. Learning, motivation, and cognition: the functional behaviorism of Robert C Bolles, Washington, DC: American Psychological Association, 1997. pp. 53.
[86] Richardson R, Wang P, Campbell BA. Delayed development of conditioned heart rate responses to auditory stimuli in the rat. Dev Psychobiol 1995;28:221–238.
[87] Supple Jr WF, Leaton RN. Cerebellar vermis: essential for classically conditioned bradycardia in the rat. Brain Res 1990;509:17–23.
[88] Iwata J, LeDoux JE. Dissociation of associative and nonassociative concomitants of classical fear conditioning in the freely behaving rat. Behav Neurosci 1988;102:66–76.
[89] Chance WT, Krynock GM, Rosecrans JA. Antinociception following lesion-induced hyperemotionality and conditioned fear. Pain 1978;4:243–252.
[90] Fanselow MS, Baackes MP. Conditioned fear-induced opiate analgesia on the formalin test: evidence for two aversive motivational systems. Learn Motiv 1982;13:200–221.
[91] Bolles RC. Species-specific defensive reactions and avoidance learning. Psychol Rev 1970;71:32–48.
[92] Fanselow MS. Neural organization of the defensive behavior system responsible for fear. Psychol Bull Rev 1994;1:429–438.
[93] Brown S, Schaffer A. An investigation into the functions of the occipital and temporal lobes of the monkey's brain. Trans R Soc London 1886;179:303–327.
[94] Kluver H, Bucy PC. Preliminary analysis of the temporal lobes in monkeys. Biol Psychiatry 1939;42:461–471.
[95] Weiskrantz L. Behavioral changes associated with ablations of the amygdaloid complex in monkeys. J Comp Physiol Psychol 1956;49:381–391.
[96] Fuster JM, Uyeda AA. Reactivity of limbic neurons of the monkey to appetitive and aversive signals. Electroencephalogr Clin Neurophysiol 1971;30:281–293.
[97] Applegate CD, Frysinger RC, Kapp BS, Gallagher M. Multiple unit activity recorded from amygdala central nucleus during Pavlovian heart conditioning in rabbit. Brain Res 1982;238:457–462.
[98] Quirk GJ, Repa JC, LeDoux JE. Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. Neuron 1995;15:1029–1039.
[99] Romanski LM, Clugnet MC, Bordi F, LeDoux JE. Somatosensory and auditory convergence in the lateral nucleus of the amygdala. Behav Neurosci 1993;107:444–450.
[100] Chapman PF, Kairiss EW, Keenan CL, Brown TH. Long-term synaptic potentiation in the amygdala. Synapse 1990;6:271–278.
[101] Clugnet MC, LeDoux JE. Synaptic plasticity in fear conditioning circuits: induction of LTP in the lateral nucleus of the amygdala by stimulation of the medial geniculate body. J Neurosci 1990;10:2818–2824.
[102] Huang YY, Kandel ER. Postsynaptic induction and PKA-dependent expression of LTP in the lateral amygdala. Neuron 1998;21:169–178.
[103] Maren S, Fanselow MS. Synaptic plasticity in the basolateral

amygdala induced by hippocampal formation stimulation in vivo. J Neurosci 1995;15:7548–7564.
[104] Li XF, Phillips RG, LeDoux JE. NMDA and non-NMDA receptors contribute to synaptic transmission between the medial geniculate body and the lateral nucleus of the amygdala. Exp Brain Res 1995;105:87–100.
[105] McKernan MG, Shinnick-Gallagher P. Fear conditioning induces a lasting potentiation of synaptic currents in vivo. Nature 1997;390:607–611.
[106] Rogan MT, Stäubli UV, LeDoux JE. Fear conditioning induces associative long-term potentiation in the amygdala. Nature 1997;390:604–607.
[107] Berg WK, Davis M. Associative Learning modifies startle reflexes at the lateral lemniscus. Behav Neurosci 1985;99:191–199.
[108] Davis M. Pharmacological and anatomical analysis of fear conditioning using the fear-potentiated startle paradigm. Behav Neurosci 1986;100:814–824.
[109] Hitchcock JM, Davis M. Lesions of the amygdala but not of the cerebellum or red nucleus block conditioned fear as measured with the potentiated startle paradigm. Behav Neurosci 1986;100:11–22.
[110] Hitchcock JM, Davis M. Fear-potentiated startle using an auditory conditioned stimulus: effect of lesions of the amygdala. Physiol Behav 1987;39:403–408.
[111] Gallagher M, Kapp BS, Pascoe JP, Rapp PR. A neuropharmacology of amygdala systems which contribute to learning and memory. In: Ben-Ari Y, editor. The amygdaloid complex, Amsterdam: Elsevier, 1981. pp. 343.
[112] Thompson RF. The neurobiology of learning and memory. Science 1986;233:941–947.
[113] Rosen JB, Hitchcock JM, Sananes CB, Miserendino MJD, Davis M. A direct projection from the central nucleus of the amygdala to the acoustic startle pathway: anterograde and retrograde tracing studies. Behav Neurosci 1991;105:817–825.
[114] Hitchcock JM, Davis M. The efferent pathway of the amygdala involved in conditioned fear as measured with the fear-potentiated startle paradigm. Behav Neurosci 1991;105:826–842.
[115] Rosen JB, Davis M. Enhancement of acoustic startle by electrical stimulation of the amygdala. Behav Neurosci 1988;102:195–202.
[116] Rosen JB, Davis M. Temporal characteristics of enhancement of startle by stimulation of the amygdala. Physiol Behav 1988;44:117–123.
[117] Rosen JB, Davis M. Enhancement of electrically elicited startle by amygdaloid stimulation. Physiol Behav 1990;48:343–349.
[118] Koch M, Ebert U. Enhancement of the acoustic startle response by stimulation of an excitatory pathway from the central amygdala/basal nucleus of Meynert to the pontine reticular formation. Exp Brain Res 1993;93:231–241.
[119] Koch M. Microinjections of the metabotropic glutamate receptor agonist, trans-(+/−)-1-amino-cyclopentane-1,3-dicarboxylate (trans-ACPD) into the amygdala increase the acoustic startle response of rats. Brain Res 1993;629:176–179.
[120] Campeau S, Miserendino MJD, Davis M. Intra-amygdaloid infusion of the *N*-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. Behav Neurosci 1992;106:569–574.
[121] Melia KR, Falls WA, Davis M. Involvement of pertussis toxin sensitive G-proteins in conditioned fear-potentiated startle: possible involvement of the amygdala. Brain Res 1992;584:141–148.
[122] Miserendino MJD, Sananes CB, Melia KR, Davis M. Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. Nature 1990;345:716–718.
[123] Falls WA, Miserendino MJD, Davis M. Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. J Neurosci 1992;12:854–863.
[124] Gewirtz JC, Davis M. Second-order fear conditioning prevented by blocking NMDA receptors in amygdala. Nature 1997;388:471–474.
[125] Tischler MD, Davis M. A visual pathway that mediates fear-conditioned enhancement of acoustic startle. Brain Res 1983;276:55–71.
[126] Campeau S, Davis M. Involvement of subcortical and cortical afferents to the lateral nucleus of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. J Neurosci 1995;15:2312–2327.
[127] Campeau S, Davis M. Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. J Neurosci 1995;15:2301–2311.
[128] Falls WA, Davis M. Visual cortex ablations do not prevent extinction of fear-potentiated startle using a visual conditioned stimulus. Behav Neural Biol 1993;60:259–270.
[129] Rosen JB, Hitchcock JM, Miserendino MJD, Falls WA, Campeau S, Davis M. Lesions of the perirhinal cortex but not of the frontal medial pre-frontal visual or insular cortex block fear-potentiated startle using a visual conditioned stimulus. J Neurosci 1992;12:4624–4633.
[130] Fendt M, Koch M, Schnitzler HU. Amygdaloid noradrenaline is involved in the sensitization of the acoustic startle response in rats. Pharmacol Biochem Behav 1994;48:307–314.
[131] Chiang C, Aston-Jones G. Response of locus coeruleus neurons to footshock stimulation is mediated by neurons in the rostral ventral medulla. Neuroscience 1993;53:705–715.
[132] Galvez R, Mesches MH, McGaugh JL. Norepinephrine release in the amygdala in response to footshock stimulation. Neurobiol Learn Mem 1996;66:253–257.
[133] Quirarte GL, Galvez R, Roozendaal B, McGaugh JL. Norepinephrine release in the amygdala in response to footshock and opioid peptidergic drugs. Brain Res 1998;808:134–140.
[134] Fallon JH. Histochemical characterization of dopaminergic, noradrenergic and serotonergic projections to the amygdala. In: Ben-Ari Y, editor. The amygdaloid complex, Amsterdam: Elsevier, 1981. pp. 175.
[135] Bernard JF, Besson JM. The spino(trigemino)pontoamygdaloid pathway: electrophysiological evidence for an involvement in pain processes. J Neurophysiol 1990;63:473–490.
[136] Davis M, Rainnie D, Cassell M. Neurotransmission in the rat amygdala related to fear and anxiety. Trends Neurosci 1994;17:208–214.
[137] Roberts GW. Neuropeptides: cellular morphology, major pathways, and functional considerations. In: Aggleton JP, editor. The amygdala: neurobiological aspects of emotion, memory, and mental dysfunction, New York: Wiley-Liss, 1992. pp. 115.
[138] McDonald AJ. Cell types and intrinsic connections of the amygdala. In: Aggleton JP, editor. The amygdala: neurobiological aspects of emotion, memory, and mental dysfunction, New York: Wiley-Liss, 1992. pp. 67.
[139] Shi C, Davis M. Anatomical tracing and lesion studies of pain pathways involved in fear conditioning measured with fear potentiated startle. Soc Neurosci Abstr 1997;23:627.12.
[140] Shi C, Davis M. Pain pathways involved in fear conditioning measured with fear-potentiated startle: lesion studies. J Neurosci 1999;19:420–430.
[141] Kim M, Campeau S, Falls WA, Davis M. Infusion of the non-NMDA receptor antagonist CNQX into the amygdala blocks the expression of fear-potentiated startle. Behav Neural Biol 1993;59:5–8.
[142] Walker DL, Davis M. Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. J Neurosci 1997;17:000.
[143] Frankland PW, Josselyn SA, Bradwejn J, Vaccarino FJ, Yeomans JS. Activation of amygdala cholecystokinin$_B$ receptors potentiates the acoustic startle response in the rat. J Neurosci 1997;17:1838–1847.
[144] Frankland PW, Josselyn SA, Vaccarino FJ, Yeomans JS, Bradwejn J. Intracerebroventricular infusion of the CCK$_B$ receptor agonist

pentagastrin potentiates acoustic startle. Brain Res 1996;733:129–132.
[145] Fendt M, Koch M, Schnitzler HU. Corticotropin-releasing factor is involved in the expression of fear-potentiated startle: an anatomical electrophysiological and behavioral study. Eur J Neurosci 1997;9:299–305.
[146] Koch M, Ebert U. Enhancement of auditory responses of pontine reticular brainstem neurons by glutamate and stimulation of the amygdaloid complex. Eur J Neurosci 1992;5:142.
[147] Fendt M, Koch M, Schnitzler HU. NMDA receptors in the pontine brainstem are necessary for fear-potentiation of the startle response. Eur J Pharmacol 1996;318:1–6.
[148] Ebert U, Koch M. Glutamate receptors mediate acoustic input to the reticular brain stem. NeuroReport 1992;3:429–432.
[149] Krase W, Koch M, Schnitzler HU. Glutamate antagonists in the reticular formation reduce the acoustic startle response. NeuroReport 1993;4:13–16.
[150] Miserendino MJD, Davis M. NMDA and non-NMDA antagonists infused into the nucleus reticularis pontis caudalis depress the acoustic startle reflex. Brain Res 1993;623:215–222.
[151] Birnbaum SG, Davis M. Modulation of the acoustic startle reflex by infusion of corticotropin-releasing hormone into the nucleus reticularis pontis caudalis. Brain Res 1998;782:318–323.
[152] Falls WA, Davis M. Inhibition of fear-potentiated startle can be detected after the offset of a feature trained in a serial feature-negative discrimination. J Exp Psychol: Animal Behav Proc 1997;23:3–14.
[153] Falls WA, Davis M. Lesions of the central nucleus of the amygdala block conditioned excitation but not conditioned inhibition of fear as measured with the fear-potentiated startle effect. Behav Neurosci 1995;109:379–387.
[154] Iwata J, LeDoux JE, Meeley MP, Arneric S, Reis DJ. Intrinsic neurons in the amygdaloid field projected to by the medial geniculate body mediate emotional responses conditioned to acoustic stimuli. Brain Res 1986;383:195–214.
[155] Kapp BS, Frysinger RC, Gallagher M, Haselton JR. Amygdala central nucleus lesions: effect on heart rate conditioning in the rabbit. Physiol Behav 1979;23:1109–1117.
[156] Helmstetter FJ. Contribution of the amygdala to learning and performance of conditional fear. Physiol Behav 1992;51:1271–1276.
[157] Watkins LR, Wiertelak EP, Maier SF. The amygdala is necessary for the expression of conditioned but not unconditioned analgesia. Behav Neurosci 1993;107:402–405.
[158] Goldstein LE, Rasmusson AM, Bunney BS, Roth RH. Role of the amygdala in the coordination of behavioral neuroendocrine and prefrontal cortical monoamine responses to psychological stress in the rat. J Neurosci 1996;16:4787–4798.
[159] Fanselow MS, Kim JJ. Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phoshonovaleric acid to the basolateral amygdala. Behav Neurosci 1994;108:210–212.
[160] Lee H, Kim JJ. Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. J Neurosci 1998;18:8444–8454.
[161] Maren S, Aharanov G, Stote DL, Fanselow MS. *N*-Methyl-D-aspartate receptors in the basolateral amygdala are required for both acquisition and expression and expression of conditioned fear in rats. Behav Neurosci 1996;110:1365–1375.
[162] Helmstetter FJ. Stress-induced hypoalgesia and defensive freezing are attenuated by application of diazepam to the amygdala. Pharmacol Biochem Behav 1993;44:433–438.
[163] Helmstetter FJ, Bellgowan PS. Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. Behav Neurosci 1994;108:1005–1009.
[164] Muller J, Corodimas KP, Fridel Z, LeDoux JE. Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. Behav Neurosci 1997;111:683–691.
[165] Armony JL, Quirk GJ, LeDoux JE. Differential effects of amygdala lesions on early and late plastic components of auditory cortex spike trains during fear conditioning. J Neurosci 1998;18:2592–2601.
[166] Quirk GJ, Armony JL, LeDoux JE. Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. Neuron 1997;19:613–624.
[167] Romanski LB, LeDoux JE. Equipotentially of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning. J Neurosci 1992;12:4501–4509.
[168] Fanselow MS. Associative vs. topographical accounts of the immediate shock freezing deficit in rats: implications for the response selection rules governing species specific defensive reactions . Learn Motiv 1986;17:16–39.
[169] Kim JJ, Fanselow MS. Modality-specific retrograde amnesia of fear. Science 1992;256:675–677.
[170] Cruikshank SJ, Edeline JM, Weinberger NM. Stimulation at a site of auditory-somatosensory convergence in the medial geniculate nucleus is an effective unconditioned stimulus for fear conditioning. Behav Neurosci 1992;106:471–483.
[171] LeDoux JE, Ruggiero DA, Forest R, Stornetta R, Reis DJ. Topographic organization of convergent projections to the thalamus from the inferior colliculus and spinal cord in the rat. J Comp Neurol 1987;264:123–146.
[172] Edeline JM, Weinberger NM. Associative retuning in the thalamic source of input to the amygdala and auditory cortex: receptive field plasticity in the medial division of the medial geniculate body. Behav Neurosci 1992;106:81–105.
[173] Fanselow MS, Kim JJ. The benzodiazepine inverse agonist DMCM as an unconditional stimulus for fear-induced analgesia: Implications for the role of GABAA receptors in fear-related behavior. Behav Neurosci 1992;106:336–344.
[174] DeLorey TM, Handforth A, Anagnostaras SG, Homanics GE, Minassian BA, Asatourian A, Fanselow MS, Delgado-Escueta A, Ellison GD, Olsen RW. Mice lacking the β_3 subunit of the $GABA_A$ receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. J Neurosci 1998;18:8505–8514.
[175] Maren S, Aharanov G, Fanselow MS. Retrograde abolition of conditioned fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. Behav Neurosci 1996;110:718–726.
[176] Helmstetter FJ. The amygdala is essential for the expression of conditioned hypoalgesia. Behav Neurosci 1992;106:518–528.
[177] Bechara A, Tranel D, Damasio H, Adolphs R, Rockland C, Damasio AR. Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. Science 1995;269:1115–1118.
[178] LaBar KS, LeDoux JE, Spencer DD, Phelps EA. Impaired fear conditioning following unilateral temporal lobectomy in humans. J Neurosci 1995;10:6846–6855.
[179] LeDoux JE, Iwata J, Cicchetti P, Reis DJ. Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. J. Neurosci. 1988;8:2517–2529.
[180] Kapp BS, Gallagher M, Underwood MD, McNall CL, Whitehorn D. Cardiovascular responses elicited by electrical stimulation of the amygdala central nucleus in the rabbit. Brain Res 1982;234:251–262.
[181] Fendt M. Stimulation of startle modulating midbrain areas has different effects on the acoustic startle response. Soc Neurosci Abstr 1997;23:627.14.
[182] Fendt M. Different regions of the periaqueductal grey are involved differently in the expression and conditioned inhibition of fear-potentiated startle. Eur J Neurosci 1998;10:3876–3884.
[183] Fendt M, Koch M, Schnitzler HU. Lesions of the central gray block

the sensitization of the acoustic startle response in rats. Brain Res 1994;661:163–173.
[184] Bandler R, Shipley MT. Columnar organization in the midbrain periaqueductal gray: modules for emotional expression. Trends Neurosci 1994;17:379–389.
[185] Carrive P, Leung P, Harris JA, Paxinos G. Conditioned fear to context is associated with increased fos expression in the caudal ventrolateral region of the midbrain periaqueductal gray. Neuroscience 1997;78:165–177.
[186] Cassella JV, Davis M. Sensitization fear conditioning and pharmacological modulation of acoustic startle following lesions of the dorsal periaqueductal gray. Soc Neurosci Abstr 1984;10:1067.
[187] Borszcz GS, Cranney J, Leaton RN. Influence of long-term sensitization on long-term habituation of the acoustic startle response in rats: Central gray lesions preexposure and extinction. J Exp Psychol: Animal Behav Proc 1989;15:54–64.
[188] Walker DL, Davis M. Involvement of the dorsal periaqueductal gray in the loss of fear-potentiated startle accompanying high footshock training. Behav Neurosci 1997;111:692–702.
[189] Borowski TB, Kokkinidis L. Contribution of ventral tegmental area dopamine neurons to expression of conditional fear: effects of electrical stimulation excitotoxin lesions and quinpirole infusion on potentiated startle in rats. Behav Neurosci 1996;110:1349–1364.
[190] Fendt M, Koch M, Schnitzler HU. Somatostatin in the pontine reticular formation modulates fear- potentiation of the acoustic startle response: an anatomical electrophysiological and behavioral study . J Neurosci 1996;16:3097–3103.
[191] Liebman JM, Mayer DJ, Liebeskind JC. Mesencephalic central gray lesions and fear-motivated behavior in rats. Brain Res 1970;23:353–370.
[192] De Oca BM, DeCola JP, Maren S, Fanselow MS. Distinct regions of the periaqueductal gray are involved in the acquisition and expression of defensive responses. J Neurosci 1998;18:3426–3432.
[193] Fanselow MS, DeCola JP, De Oca B, Landeira-Fernandez J. Ventral and dorsolateral regions of the midbrain periaqueductal gray (PAG) control different stages of defensive behavior: dorsolateral PAG lesions enhance the defensive freezing produced by massed and immediate shock. Aggress Behav 1995;21:61–77.
[194] Maier SF, Grahn RE, Kalman BA, Sutton LC, Wiertelak EP, Watkins LR. The role of the amygdala and dorsal raphe nucleus in mediating the behavioral consequences of inescapable shock. Behav Neurosci 1993;107:377–388.
[195] Bandler R, Depaulis A. Midbrain periaqueductal gray control of defensive behavior in the cat and the rat. In: Depaulis A, Bandler R, editors. The midbrain periaqueductal gray, New York: Plenum Press, 1991 pp. 175–198.
[196] Helmstetter FJ, Landeira-Fernandez J. Conditioned hypoalgesia is attenuated by naltrexone applied to the periaqueductal gray. Brain Res 1990;537:88–92.
[197] Krase W, Koch M, Schnitzler HU. Substance P is involved in the sensitization of the acoustic startle response by footshocks in rats. Behav Brain Res 1994;63:81–88.
[198] Kungel M, Ebert U, Herbert H, Ostwald J. Substance P and other putative transmitters modulate the activity of reticular pontine neurons: an electrophysiological and immunohistochemical study. Brain Res 1994;643:29–39.
[199] Frankland PW, Yeomans JS. Fear-potentiated startle and electrically evoked startle mediated by synapses in rostrolateral midbrain. Behav Neurosci 1995;109:669–680.
[200] Fendt M, Koch M, Kungel M, Schnitzler HU. Cholecystokinin enhances the acoustic startle response in rats. NeuroReport 1995;6:2081–2084.
[201] Vanderhaeghen JJ. Neuronal cholecystokinin. In: Björklund A, Hökfelt T, editors. Handbook of chemical neuroanatomy. Vol. 4: GABA and Neuropeptides in the CNS, Amsterdam: Elsevier, 1985. pp. 406 Part I.
[202] O'Keefe J, Nadel L. The hippocampus as a cognitive map. New York: Oxford University Press, 1978.
[203] Sutherland RJ, Rudy JW. Configural association theory: the role of the hippocampal formation in learning memory and amnesia. Psychobiology 1989;17:129–144.
[204] Kim JJ, Rison RA, Fanselow MS. Effects of amygdala hippocampus and periaqueductal gray lesions on short- and long-term contextual fear. Behav Neurosci 1994;107:1088–1092.
[205] Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 1992;106:274–285.
[206] Maren S, Aharanov G, Fanselow MS. Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. Behav Brain Res 1997;88:261–274.
[207] Maren S, Anagnostaras SG, Fanselow MS. The startled seahorse: Is the hippocampus necessary for contextual fear conditioning. Psychol Rev 1998;2:195–231.
[208] Maren S, Fanselow MS. Electrolytic lesions of the fimbria/fornix dorsal hippocampus or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats. Neurobiol Learn Memory 1997;67:142–149.
[209] Squire LR. Memory and the hippocampus: A synthesis from findings with rats monkeys and humans. Psychol Rev 1992;2:195–231.
[210] Anagnostaras SG, Maren S, Fanselow MS. Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-examination. J Neurosci 1999;19:1106–1114.
[211] McNish KA, Gewirtz JC, Davis M. Evidence of contextual fear after lesions of the hippocampus: a disruption of freezing but not fear-potentiated startle. J Neurosci 1997;17:9353–9360.
[212] Coover GD, Levine ES. Auditory startle response of hippocampectomized rats. Physiol Behav 1972;9:75–77.
[213] Graeff FG. Neuroanatomy and neurotransmitter regulation of defensive behaviors and related emotions in mammals. Brazilian J Med Biol Res 1994;27:811–829.
[214] Lavond DG, Kim JJ, Thompson RF. Mammalian brain substrates of aversive classical conditioning. Annu Rev Psychol 1993;44:317–342.
[215] LeDoux JE. Emotion: clues from the brain. Annu Rev Psychol 1995;46:209–235.
[216] Maren S, Fanselow MS. The amygdala and fear conditioning: has the nut been cracked. Neuron 1996;16:237–240.
[217] Collingridge GL, Bliss TVP. NMDA receptors—their role in long-term potentiation. Trends Neurosci 1987;10:288–293.
[218] Rison RA, Stanton PK. Long-term potentiation and *N*-methyl-D-aspartate receptors: foundations of memory and neurological disease. Neurosci Biobehav Rev 1995;19:533–552.
[219] Rogan MT, LeDoux JE. LTP is accompanied by commensurate enhancement of auditory-evoked responses in a fear conditioning circuit. Neuron 1995;15:127–136.
[220] Teich AH, McCabe PM, Gentile CG, Jarrell TW, Winters RW, Liskowsky DR, Schneiderman N. Role of the auditory cortex in the acquisition of differential heart rate conditioning. Physiol Behav 1988;44:405–412.
[221] Neff WD, Diamond IT. The neural basis of auditory discrimination. In: Harlow HF, Woosley CN, editors. Biological and biochemical bases of behavior, Madison: University of Wisconsin Press, 1958. pp. 101.
[222] Thompson RF. Function of the auditory cortex of cat in frequency discrimination. J Neurophysiol 1960;23:321–334.
[223] Whitfield IC, Cranford J, Ravizza R, Diamond IT. Effects of unilateral ablation of auditory cortex in cat on complex sound localization. J Neurophysiol 1972;35:718–731.
[224] Davis M, Schlesinger LS, Sorenson CA. Temporal specificity of fear conditioning: effects of different conditioned stimulus - unconditioned stimulus intervals on the fear-potentiated startle effect. J Exp Psychol: Animal Behav Proc 1989;15:295–310.
[225] Faulkner B, Tieu KH, Brown TH. Mechanism for temporal encoding

in fear conditioning: delay lines in perirhinal cortex and lateral amygdala. In: Bower G, editor. Computational neuroscience, New York: Plenum Press, 1997. pp. 641.

[226] Li XF, Stutzmann GE, LeDoux JE. Convergent but temporally separated inputs to lateral amygdala neurons from the auditory thalamus and auditory cortex use different postsynaptic receptors: in vivo intracellular and extracellular recordings in fear conditioning pathways. Learn Mem 1996;3:229–242.

[227] Kim M, Davis M. Electrolytic lesions of the amygdala block acquisition and expression of fear-potentiated startle even with extensive training but do not prevent reacquisition. Behav Neurosci 1993;107:580–595.

[228] Maren S. Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. J Neurosci 1998;18:3088–3097.

[229] Killcross SS, Robbins TW, Everitt BJ. Different types of fear-conditioned behavior mediated by separate nuclei within the amygdala. Nature 1997;388:77–380.

[230] Moye TB, Rudy JW. Ontogenesis of learning. VI. Learned and unlearned responses to visual stimulation in the infant hooded rat . Dev Psychobiol 1985;18:887–891.

[231] Rudy JW. Contextual and auditory cue conditioning dissociate during development. Behav Neurosci 1993;107:887–891.