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The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction

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Abstract

Using a paradigm known as fear-potentiated startle, we have examined the neurobiological substrates of Pavlovian fear conditioning. In these experiments, rats are trained to fear an initially neutral stimulus by pairing that stimulus with shock. The amount of fear elicited by the stimulus [i.e., now a conditioned stimulus (CS)] is later assessed by presenting startle-eliciting noise bursts both in the presence and also the absence of the CS. After training, startle responses are typically greater in the presence of the CS. Findings reviewed here suggest that amygdala *N*-methyl-D-aspartate (NMDA) receptors play a key role in triggering the neural changes that support fear learning and also the loss of fear that accompanies extinction training. Amygdala (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors also participate in fear learning. However, unlike NMDA receptor antagonists, AMPA receptor antagonists also block fear-potentiated startle when infused prior to testing. Very recent data indicate that glutamate metabotropic Group II receptor agonists also block fear learning when infused into the amygdala prior to training, and block fear-potentiated startle when infused prior to testing. A fuller understanding of the role of amygdala glutamate systems in fear and fear learning may suggest novel pharmacological approaches to the treatment of clinical anxiety disorders.

Keywords: Glutamate; Metabotropic receptor; *N*-methyl-D-aspartate; Fear learning; Fear-potentiated startle; Anxiety; Amygdala; D-Cycloserine

1. Introduction

The amygdala is a group of spatially contiguous and anatomically interconnected nuclei located within the rostral pole of the temporal lobe of mammals. A prominent role for the amygdala in the evaluation of biologically significant stimuli and in the generation of responses to such stimuli has long been recognized, and has been particularly well documented with respect to fear-evoking stimuli (e.g., Blanchard and Blanchard, 1972; Goddard, 1964; Robinson, 1963; Slotnick, 1973). In recent years, it also has become evident that the amygdala plays an important role in fear learning (e.g., Davis, 2000; Fendt and Fanselow, 1999)

For several years now, we have examined the role of the amygdala in fear and fear learning using fear-potentiation of the acoustic startle reflex as a behavioral measure. For these experiments, rats are trained by pairing a brief initially neutral stimulus (most often a 3.7-s light, although tones and olfactory stimuli have also been used) with a 0.5-s footshock unconditioned stimulus (US). Rats are later tested by presenting them with a series of startle-eliciting noise bursts. Some of these noise bursts are presented in the presence of the stimulus that had previously been paired with shock, while others are presented in its absence. Fear-potentiated startle is defined as an increase in startle amplitude in the presence versus the absence of the conditioned fear stimulus (Fig. 1). The stimulus does not itself elicit a startle response but, instead, elicits a state of fear that potentiates startle responses to other stimuli.

2. The role of the amygdala and its efferent projections in fear-potentiated startle

The brainstem circuit that mediates the primary acoustic startle response consists of three sets of synapses: those made by spiral ganglion cells within the cochlea onto

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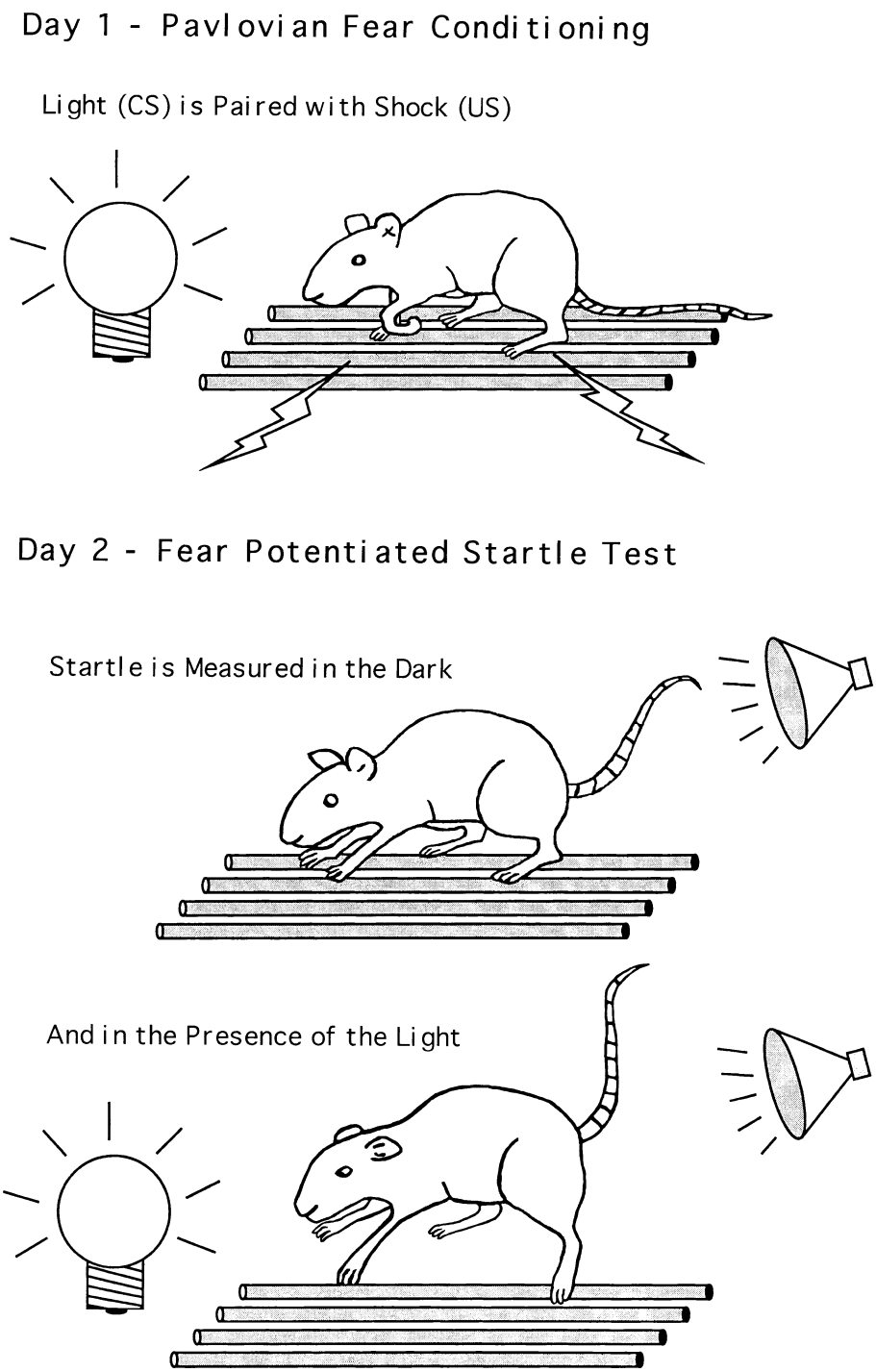

Fig. 1. During Pavlovian fear conditioning, a neutral stimulus such as a brief light (3.7 s) is paired with footshock (0.5 s). During testing, a series of startle-eliciting noise bursts are presented and the amplitude of the rats startle response to these noise bursts is recorded. Half of these noise bursts are presented in the presence of the conditioned fear stimulus (in the above example, a light) whereas half are presented in its absence. After fear conditioning, startle amplitude is typically greater on CS–noise compared to noise-alone trials.

cochlear root neurons, those made by cochlear root neurons onto neurons within the nucleus reticularis pontis caudalis (PnC), and those made by PnC neurons onto spinal motor neurons (for supporting evidence, see Davis et al., 1982; Lee et al., 1996a; Miserendino and Davis, 1993) (Fig. 2). Berg and Davis (1985) electrically elicited startle responses at several points along this serial pathway. Conditioned stimulus (CS) presentations increased the amplitude of startle responses that were evoked by electrical stimulation at sites afferent to the PnC but did not increase the amplitude of startle responses elicited by electrical stimulation at the PnC itself or at sites downstream from the PnC.

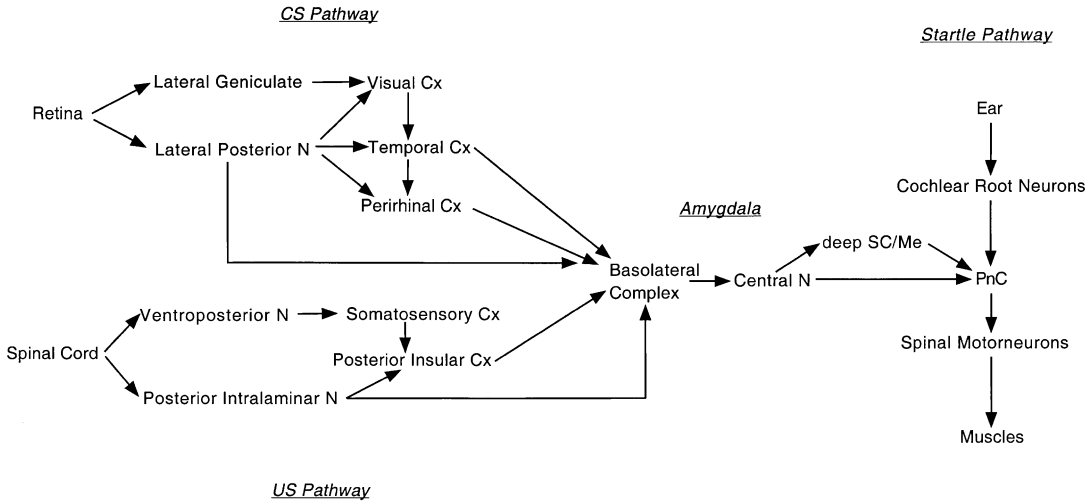

Fig. 2. Schematic summary of sensory pathways that convey CS and US information to the amygdala and their interaction with the primary startle circuit. Based on lesion and tract-tracing studies, we believe that CS and US inputs access the basolateral complex of the amygdala using parallel and redundant pathways as illustrated above. The amygdala modulates startle amplitude by way of direct and possibly indirect outputs [e.g., via the deep layers of the superior colliculus/mesencephalic reticular formation (deep SC/Me)] to the PnC — an obligatory relay within the brainstem circuitry that mediates the primary acoustic startle reflex.

These findings suggested that the modulatory influence of CS-elicited fear inserted into the primary acoustic startle circuit at the level of the PnC.

In 1991, Rosen et al. identified a direct monosynaptic projection from the central nucleus of the amygdala to the PnC. Soon thereafter, Hitchcock and Davis (1991) demonstrated that transections of the caudal extension of the ventro-amygdalafugal pathway — the fiber bundle that connects the central nucleus of the amygdala with the PnC — abolished fear-potentiated startle without significantly influencing baseline startle. More recent studies have shown that chemical lesions of the central gray (Fendt et al., 1996) and areas just lateral to the central gray (Frankland and Yeomans, 1995) — regions through which the ventro-amygdalafugal pathway passes on its way to the startle circuit — also block fear-potentiated startle. The use of fiber-sparing lesions in Fendt et al. (1996) and in Frankland and Yeomans (1995) suggest the existence of polysynaptic connections between the central nucleus of the amygdala and the PnC, in addition to the monosynaptic component identified by Rosen et al. Evidence for a polysynaptic component is also supported by findings from tract-tracing (Fendt et al., 1994) and electrical collision (Franklin and Yeomans, 1995) experiments, and the fact that local infusion of the $GABA_A$ agonist, muscimol, into this general area totally blocks the expression but not the acquisition of fear-potentiated startle without having any effect on baseline startle (Meloni and Davis, 1999).

Posttraining electrolytic (Hitchcock and Davis, 1986) as well as excitotoxic (Campeau and Davis, 1995) lesions of the central nucleus of the amygdala (the origin of the ventro-amygdalafugal pathway) also abolish fear-potentiated startle as do pretest infusions into the central nucleus of the selective (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist, NBQX (Fig. 3A). Other studies have found that lesions of the basolateral amygdala are similarly effective (e.g., Campeau and Davis, 1995; Lee et al., 1996b; Sananes and Davis, 1992). This is not surprising insofar as the basolateral amygdala receives CS and US information and is the primary source of afferent innervation for the central nucleus of the amygdala. As with central nucleus infusions, pretest infusions of NBQX into the basolateral amygdala completely block fear-potentiated startle (Fig. 3A) (Walker and Davis, 1997b). The disruptive effect of AMPA receptor blockade has been demonstrated using visual (Kim et al., 1993; Walker and Davis, 1997b), auditory (Kim et al., 1993), and also olfactory (Walker et al., unpublished observations) cues as conditioned fear stimuli (Fig. 3B).

Very recent data from our laboratory have also implicated amygdala Group II metabotropic receptors in fear and fear learning. Pretest infusions into the basolateral amygdala of the metabotropic Group II receptor agonist, LY354740, significantly disrupted fear-potentiated startle (Fig. 4A) (Stanek et al., 2000). When later tested without drug, these same rats showed normal fear-potentiated startle, indicating that the previously observed disruption of fear-potentiated startle was not attributable to permanent amygdala damage. The effect of LY354740 was mimicked by the structurally dissimilar Group II agonist, APDC, and was reversed by systemic administration of the Group II antagonist, LY341495 (Fig. 4A).

In addition to effects on expression, intra-amygdala infusions of LY354740 also disrupted fear learning (pretraining infusions) (Fig. 4B). For acquisition as well as expression, the effects of LY354740 were only apparent in rats that received bilateral amygdala infusions. Rats with cannula implanted just outside of the amygdala (average

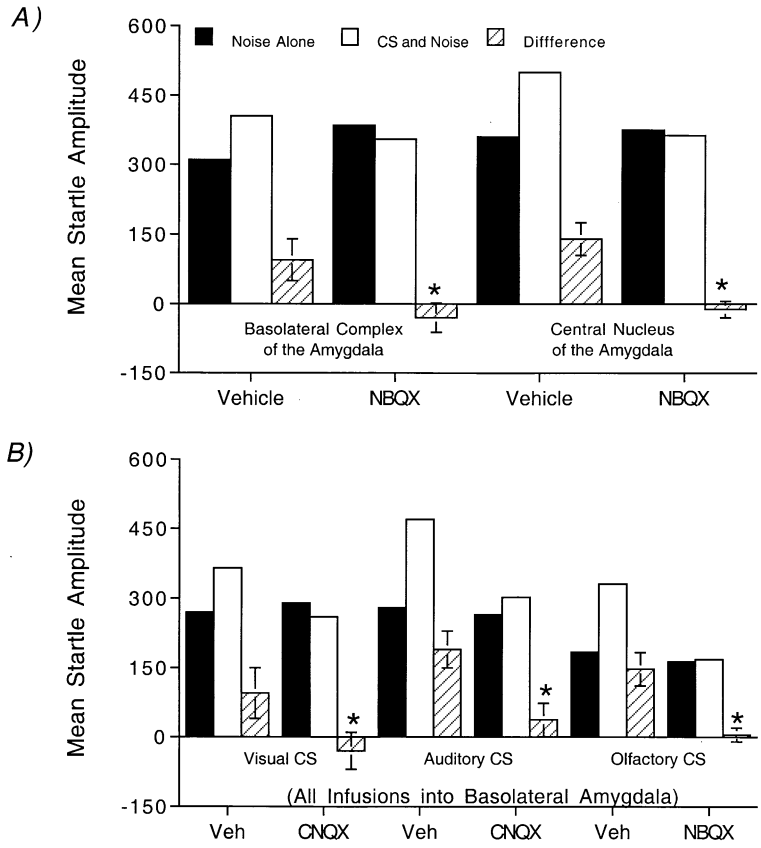

Fig. 3. Infusions of the selective AMPA receptor antagonist, NBQX, block fear-potentiated startle to visual CSs when infused into either the basolateral complex of the amygdala or the central nucleus of the amygdala (panel A). The effects of non-NMDA ionotropic glutamate receptor blockade are modality-independent. Infusions into the basolateral amygdala of either NBQX or CNQX have been found in separate studies to disrupt fear-potentiated startle to visual, auditory, and olfactory CSs (panel B). Mean startle amplitude (arbitrary units) on noise-alone and CS–noise trials are shown, along with the difference in startle amplitude between these two trial types (striped bar ± S.E.M.). Asterisks indicate statistical significance ($P < .05$) versus vehicle controls.

miss = 1.2 mm) showed only minimal impairments (Fig. 4B). The effects on both acquisition and expression could not readily be attributed to state-dependency insofar as comparable impairments were noted in animals that received LY354740 infusions prior to both training and testing (Fig. 4B).

The anxiolytic actions of intra-amygdala infusions replicate the effect of systemic administration (Helton et al., 1998). Although the mechanism of LY354740's anxiolytic actions are not known, stimulation of presynaptic Group II receptors has been shown to inhibit glutamate release in several systems (Kilbride et al., 1998; Schoepp, 1994), to hyperpolarize basolateral amygdala neurons (Patil and Rainnie, 2000), and to play an important role in the long-term depression of synaptic transmission in amygdala circuits (Heinbockel and Pape, 2000; Li et al., 1998; Wang and Gean, 1999). Any of these actions may be relevant to the efficacy of LY3544740 in the fear-potentiated startle paradigm and in other anxiety models (Helton et al., 1998; Klodzinska et al., 1999).

Together, these results implicate the amygdala and, in particular, glutamate receptor systems within the amygdala in the expression of fear-potentiated startle. Together with supporting anatomical findings, the findings suggest a serial flow of information from the basolateral amygdala to the central nucleus of the amygdala and out to the PnC (Fig. 2).

3. The amygdala as a critical site of plasticity for fear learning

It has previously been noted that the convergence of sensory pathways within the basolateral amygdala (e.g., Aggleton and Mishkin, 1986; LeDoux, 1990) makes this area an attractive anatomical substrate for associative fear conditioning. Lesion and anterograde tracing studies from our laboratory suggest that visual CS information reaches the amygdala via parallel inputs originating in the lateral geniculate and lateral posterior nucleus of the thalamus (Shi and Davis, unpublished findings), whereas footshock information reaches the amygdala via pathways originating in the posterior intralaminar and ventroposterior thalamic nuclei (Shi and Davis, 1999) (Fig. 2). Anatomical convergence of CS and US information is a logical requirement for fear conditioning circuitry and adds to the appeal of the

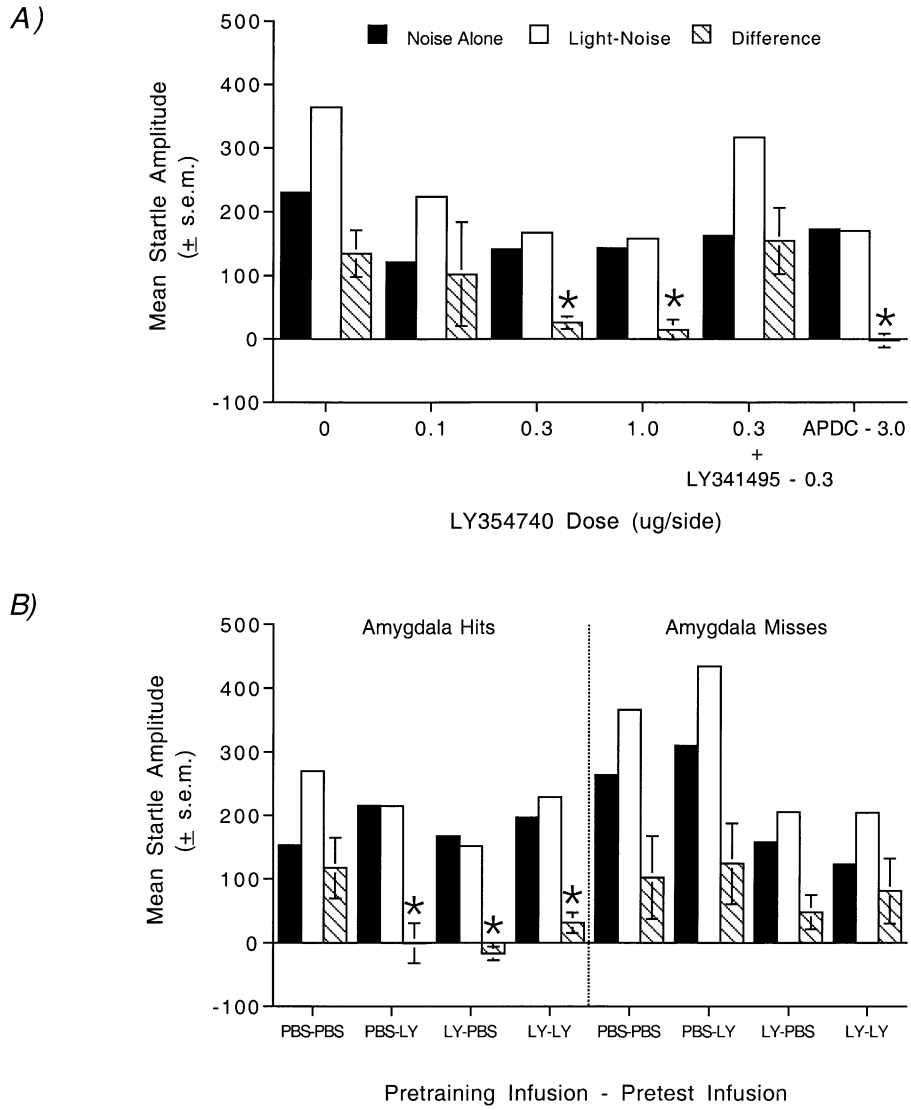

Fig. 4. The Group II metabotropic agonists, LY354740 and APDC, block fear-potentiated startle when infused into the amygdala immediately prior to testing (panel A). The Group II antagonist, LY341495, reversed the effect of LY354740. LY354740 also disrupted acquisition when infused prior to training (panel B). Neither effect could readily be attributed to state-dependence insofar as animals that received drug prior to training as well as testing were also impaired. These effects were anatomically specific in that LY354740 was significantly less effective (main ANOVA effect of Placement) in rats with one or more cannula located outside the amygdala. Mean startle amplitude on noise-alone and light–noise trials are shown, along with the difference in startle amplitude between these two trial types (striped bar ± S.E.M.). Asterisks indicate statistical significance ($P < .05$) versus 0 μg/side.

basolateral amygdala as a brain area that may contain a memory trace for fear learning. In fact, several studies have identified training-dependent changes in the electrophysiological response properties of amygdala neurons following fear conditioning (e.g., Applegate et al., 1982; Maren, 2000; Maren et al., 1991; Muramoto et al., 1993; Pascoe and Kapp, 1985; Quirk et al., 1995; 1997; Rogan et al., 1997).

Particularly relevant are findings from McKernan and Shinnick-Gallagher (1997). In this study, rats received 2 days of tone–shock pairings, or received unpaired presentations of tone and shock, or remained experimentally naïve. A subsequent test session demonstrated fear-potentiated startle only in those rats that received paired tone–shock presentations. Brain sections were then prepared from rats of each group. Whole-cell recordings of amygdala neurons from rats that received paired presentations showed a significantly greater response to stimulation of the internal capsule—a pathway that conveys auditory information to the amygdala—than did amygdala neurons from the two control groups. The difference persisted when the AMPA-mediated component of the evoked response was isolated by bath application of the *N*-methyl-D-aspartate (NMDA) receptor antagonist, AP5 (the combination of AP5 and an AMPA receptor antagonist, GYKI 52466, abolished the evoked response completely), indicating that the increased response to internal capsule stimulation was mediated primarily by a modification of AMPA receptor-mediated transmission.

These findings are consistent with previously mentioned behavioral results showing that intra-amygdala infusions of AMPA receptor antagonists block the expression of fear-potentiated startle (Kim et al., 1993; Walker and Davis, 1997b). These results suggest further that the amygdala is indeed a site of neural plasticity during fear conditioning, and that this learning involves a modification of glutamate-mediated transmission.

4. The involvement of amygdala NMDA receptors in fear learning

Several studies have shown that high-frequency stimulation of amygdala afferents can result in a long-term potentiation (LTP) of neurotransmission at amygdala synapses (Chapman et al., 1990; Clugnet and LeDoux, 1990; Gean et al., 1993; Huang and Kandel, 1998; Huang et al., 2000; Maren and Fanselow, 1995; Shindou et al., 1993; Watanabe et al., 1995; Yaniv and Richter-Levin, 2000; Yaniv et al., 2000). The mechanisms that underlie LTP may be similar to those engaged by fear conditioning (e.g., Rogan et al., 1997). Because the induction but not the expression of LTP most often involves NMDA receptors — an observation derived largely from hippocampal studies but one that may also hold true for some amygdala pathways (e.g., Gean et al., 1993; Maren and Fanselow, 1995; Shindou et al., 1993) — we wondered whether amygdala NMDA receptors might also play a special role in fear learning.

To examine this question, Miserendino et al. (1990) infused the NMDA antagonists D,L-AP5 (6.25, 12.5, 25, or 50 nmol/side) or AP7 (50 nmol/side) into the basolateral amygdala prior to light–shock pairings and tested animals for fear-potentiated startle 1 week later. Both compounds significantly disrupted fear learning as assessed with this measure, with the higher doses producing a nearly complete blockade (Fig. 5). The effect showed pharmacological and anatomical specificity. Infusions of the beta-adrenergic antagonist receptor antagonist, propranolol, did not disrupt fear learning, nor did infusions of a very high dose of D,L-AP5 (100 nmol/side) into the interpositus nucleus of the cerebellum (a structure previously implicated in other types of classical conditioning). In addition, intra-amygdala infusions of D,L-AP5 made 5 days after training and 1 week before testing had no effect on fear-potentiated startle. This indicated that the impairment produced by pretraining administration did not result from permanent damage to the amygdala that would have interfered with fear-potentiated startle during testing. The primary findings of this study have been replicated using auditory (Campeau et al., 1992) and, more recently, olfactory (Paschall et al., 2001) cues as conditioned fear stimuli (Fig. 6A). Thus, across multiple CS modalities, activation of NMDA receptors within the amygdala appears necessary for fear learning.

Importantly, intra-amygdala infusions of D,L-AP5, at doses that disrupt learning when given prior to training,

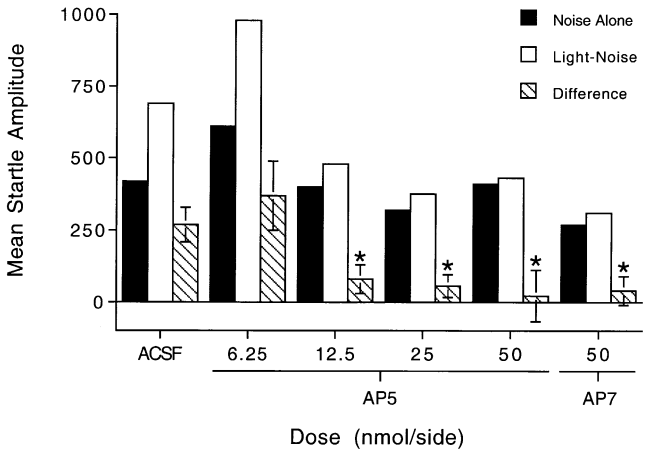

Fig. 5. Intra-amygdala infusions of the NMDA receptor antagonist, AP5, disrupt fear learning in a dose-dependent manner. Infusions of another NMDA antagonist, AP7, are similarly effective. Testing was conducted 1 week after training. Mean startle amplitude on noise-alone and light–noise trials are shown, along with the difference in startle amplitude between these two trial types (striped bar ± S.E.M.). Asterisks indicate statistical significance ($P < .05$) versus the ACSF control group.

do not disrupt the ability of conditioned fear stimuli to potentiate startle when infused prior to testing (Campeau et al., 1992; Gewirtz and Davis, 1997; Miserendino et al., 1990; Paschall et al., 2001) (Fig. 6B). Because the amygdala is, as previously indicated, essential for the expression of fear-potentiated startle (Campeau and Davis, 1995; Hitchcock and Davis, 1987; Kim et al., 1993; Sananes and Davis, 1992; Walker and Davis, 1997b), these findings indicate that the effects of NMDA receptor blockade on fear learning cannot be attributed to a general disruption of amygdala activity or to a more specific disruption of the ability of rats to process CS information. These findings also indicate that the effects on learning cannot be attributed to anxiolytic influences insofar as such influences should also disrupt fear-potentiated startle when NMDA receptor antagonists are infused prior to testing. These findings are consistent, however, with observations that NMDA-mediated currents often contribute minimally to synaptic transmission but play a more prominent role in triggering intracellular cascades such as those involved in neural plasticity.

Although the inability of pretest infusions to disrupt fear-potentiated startle indicates that the effects of pretraining infusions cannot be attributed to a failure of CS processing, it could still be argued that AP5-induced learning impairments are attributable to a disruption of US processing. We believe that this is also unlikely. Miserendino et al. (1990) reported that reactions to footshocks in vehicle and AP5-infused rats — assessed using the same procedures used to measure startle to a sudden noise — were indistinguishable, even at a dose fourfold higher than that required to significantly disrupt learning. Subsequent experiments have confirmed this finding (e.g., Campeau et al., 1992). Thus, we have found no evidence for an analgesic influence of this treatment.

More recent findings indicate that intra-amygdala D,L-AP5 infusions disrupt fear conditioning even when stimuli other than footshock are used as the reinforcing stimulus. Gewirtz and Davis (1997) reported that intra-amygdala D,L-AP5 infusions block second-order fear conditioning—a procedure in which a previously trained CS substitutes for shock as the aversive reinforcing stimulus. In this study, rats received pairings of an auditory stimulus (i.e., first-order noise CS) and footshock. On other days, the same rats were given second-order conditioning trials in which a light (i.e., the second-order CS) was paired not with shock but with the fear-eliciting first-order auditory CS. Prior to these second-order conditioning trials, rats received intra-amygdala infusions of either artificial cerebrospinal fluid (ACSF) or D,L-AP5. When subsequently tested, both groups showed fear-potentiated startle to the auditory CS. However, rats that had received D,L-AP5 did not show fear-potentiated startle to the light (Fig. 7). Because D,L-AP5 was only given prior to light–tone pairings, the ability of D,L-AP5 to block fear learning could not be attributed to analgesic actions or to a disruption of neural transmission in pathways that convey footshock information to the amygdala. Furthermore, in the same rats where AP5 blocked second-order fear conditioning using the noise as the reinforcement, AP5 did not disrupt fear-potentiated startle to the first-order noise CS (Fig. 7). In fact, it significantly increased fear-potentiated startle to the noise. These data strongly suggest that AP5 disrupted the acquisition of fear by preventing the association between light and noise, rather than by preventing amygdala activation by the noise stimulus that was used as the reinforcement in second-order conditioning.

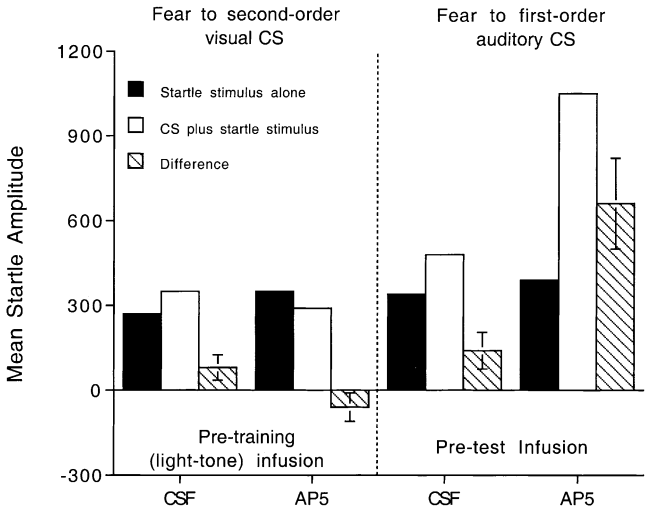

Fig. 7. Intra-amygdala D,L-AP5 infusions disrupt second-order fear conditioning. Rats were trained with tone–shock pairings (first-order conditioning) and, on other days, with light–tone pairings (second-order conditioning). Prior to light–tone pairings, rats received intra-amygdala infusions of either CSF or D,L-AP5. D,L-AP5 disrupted second-order fear conditioning (i.e., fear-potentiated startle to the light) (left panel). The same rats were later infused with D,L-AP5 prior to testing. D,L-AP5 did not disrupt the expression of fear-potentiated startle to the auditory CS (right panel). This result suggests that D,L-AP5 did not disrupt second-order fear conditioning by preventing the auditory reinforcement signal from activating amygdala neurons during light–tone pairings.

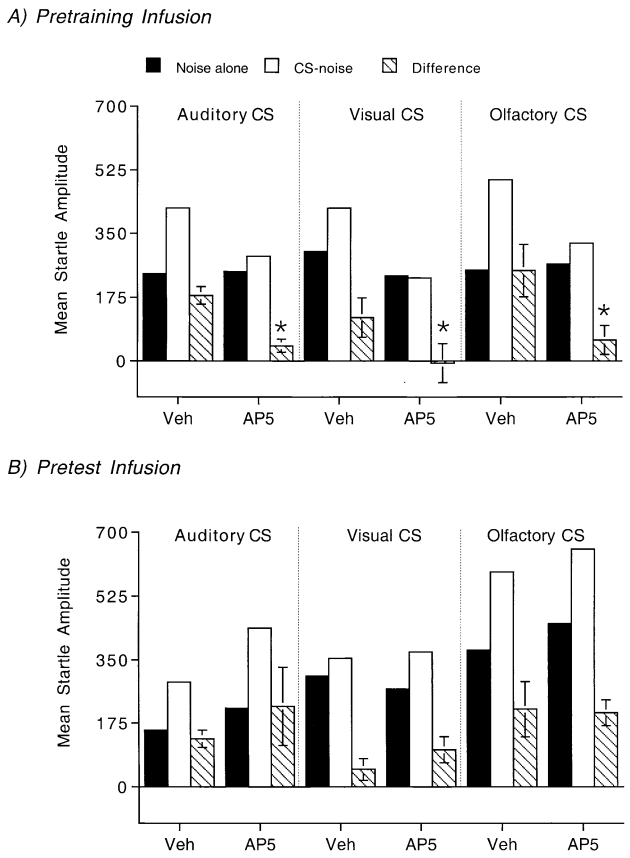

Fig. 6. Pretraining intra-amygdala infusions of the NMDA receptor antagonist, AP5 (12.5 nmol/side), disrupt fear learning across multiple CS modalities (panel A). However, pretest infusions of the same dose of AP5 do not disrupt fear-potentiated startle (panel B). Together with evidence from lesion and reversible inactivation studies indicating that amygdala inactivation prevents fear-potentiated startle, these findings indicate that AP5 does not disrupt fear learning by inactivating the amygdala. Instead, NMDA receptors within the amygdala appear to play a special role in fear learning. Asterisks indicate statistical significance ($P < .05$) versus the vehicle control group.

5. The role of amygdala NMDA receptors in short-term memory for fear learning

In all of the above studies, we have assessed fear-potentiated startle using train–test intervals of at least 24 h (i.e., long-term memory). Using these protocols, we have concluded that NMDA receptors within the amygdala play a critical role in fear learning. An alternative possibility is that NMDA receptors within the amygdala participate not in the initial acquisition of fear memories but in their retention, maintenance, or consolidation. On tests of long-term memory, it is not possible to distinguish between these possibilities. Either effect would produce a loss of fear-potentiated startle at long train–test intervals. However, as train–test

intervals become shorter, impairments of retention, maintenance, and consolidation, but not of acquisition, should become increasingly less severe. Indeed, there are several findings that are consistent with this pattern.

Kim and McGaugh (1992) examined the effect of intra-amygdala infusions of several NMDA antagonists (i.e., AP5, MK801, and CPP) on the acquisition and short-term memory of inhibitory avoidance (using a trials to criterion measure) as well as its long-term retention (using a latency to enter the shock compartment measure). For each of the compounds tested, there was no effect on the trials to criterion measure, suggesting that acquisition and short-term memory were intact. However, these same animals showed significant deficits when avoidance was assessed 48 h after training. Similar results have been reported by Bianchin et al. (1999). In that study, intra-amygdala administration of several drugs, including AP5 and the AMPA/kainate receptor antagonist CNQX, disrupted long-term memory (24 h train–test interval) in a step-down inhibitory avoidance paradigm, but had no effect on working (3 s train–test interval) or short-term (1.5 h train–test interval) memory. Is it possible that NMDA antagonists might be having a similar effect in our paradigm—disrupting the long-term stability of fear memories but not their initial acquisition?

To examine this possibility, we modified our standard paradigm to allow us to assess fear-potentiated startle immediately after training (short-term memory) as well as 48 h later (long-term memory). Using this procedure, we reexamined the effect of pretraining intra-amygdala D,L-AP5 (25 nmol/side) infusions (Walker and Davis, 2000). Despite a complete disruption of fear-potentiated startle on the long-term memory test (Fig. 7A), the effects on short-term memory were relatively modest and were not statistically significant. At face value, these results seemed to support the view that intra-amygdala infusion of NMDA receptor antagonists disrupts retention rather than acquisition in the fear-potentiated startle paradigm. However, it was also apparent that the level of fear-potentiated startle in vehicle-infused rats was considerably greater on the short-term memory test than on the long-term memory test. Thus, it was possible that the greater difficulty in disrupting short-term memory did not indicate a fundamental difference in the susceptibility of short- versus long-term memory to NMDA receptor blockade but reflected instead a greater resistance of the stronger short-term memory to disruption by any treatment.

To evaluate this possibility, an additional group of rats was trained using lower footshock levels (0.3 mA as opposed to the 0.6 mA footshock level used in the original experiment) so as to equate the magnitude of fear-potentiated startle on the short-term memory test with the level of fear-potentiated startle observed on the long-term memory test in animals trained with the higher footshock level. When the strength of conditioned fear on the short-term memory test was reduced in this manner, an effect of intra-amygdala NMDA receptor blockade was indeed apparent (Fig. 8B) and was comparable to that observed during the long-term memory test in animals trained with 0.6 mA footshocks. Thus, intra-amygdala infusions of D,L-AP5 produce comparable short- and long-term memory deficits when assessed against comparable baselines.

It is unclear whether a similar interpretation might account for the Kim and McGaugh (1992) and Bianchin

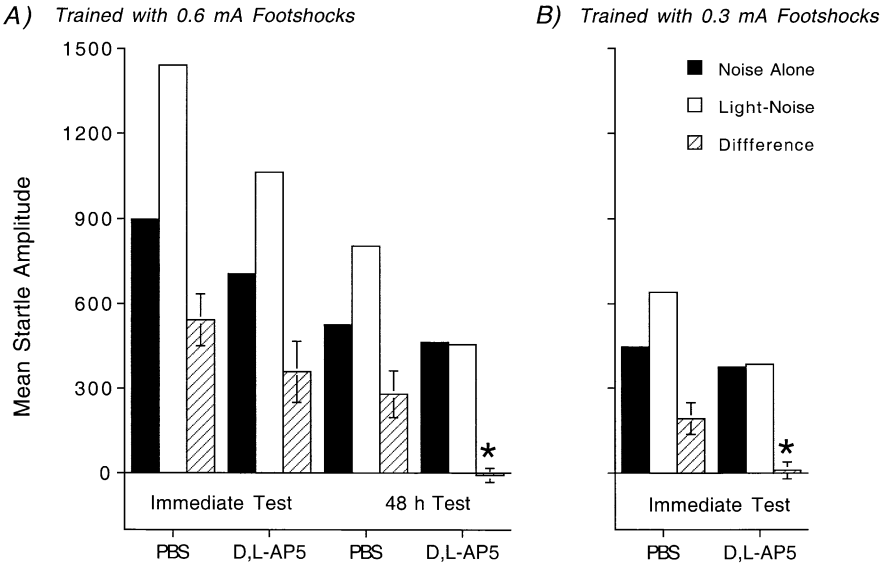

Fig. 8. Pretraining infusions of D,L-AP5 (25 nmol/side) nonsignificantly decreased fear-potentiated startle when learning was assessed immediately after training, and abolished fear-potentiated startle on a long-term memory test conducted 48 h later (panel A). When the level of fear-potentiated startle on the short-term test was lowered to that observed during the 48 h test by training animals with a lower intensity footshock (0.3 versus 0.6 mA), a significant effect of intra-amygdala NMDA blockade was observed on the short-term test as well (panel B). Thus, both short- and long-term memory are susceptible to NMDA receptor blockade. Graphs show the mean startle amplitude on noise-alone and light–noise trials, and the difference in startle amplitude between these two trial types (striped bar ± S.E.M.). Asterisks indicate statistical significance ($P < .05$) versus phosphate-buffered saline (PBS) controls.

et al. (1999) findings. However, it should be noted that there is increasing evidence to suggest that the amygdala's role in avoidance learning is different from the amygdala's role in Pavlovian fear conditioning (Amorapanth et al., 2000; Lee et al., 1996b; Liang et al., 1982; Maren et al., 1996; Wilensky et al., 2000a). With reference to the present findings, amygdala NMDA receptors may participate in the initial acquisition of Pavlovian fear memories, and participate in posttraining consolidation processes important for avoidance learning.

Taken together, we believe that the most parsimonious account of these findings is that neurons within the amygdala are modified during Pavlovian fear conditioning by an NMDA receptor-dependent process, and that these modifications are critical for the retention of learned fear. These findings do not argue that the amygdala is the only site of neuroplasticity for fear learning, only that it is a critical one.

6. Evidence for preserved learning following intra-amygdala AP5 — a possible caveat

Although these findings indicate that fear conditioning depends critically on NMDA receptors within the amygdala, the preceding experiments also provided evidence that some other form of learning related to fear conditioning survives intra-amygdala NMDA receptor blockade. At the beginning of the long-term memory test, startle amplitude to a series of 10 noise bursts was recorded in order to establish a pre-CS baseline. Afterwards, startle responses were elicited by an alternating series of noise-alone and light–noise trials. As previously stated, the difference between startle amplitude on these two trial types served as a measure of fear. Although there was no evidence for fear-potentiated startle in D,L-AP5-treated rats using this, our standard measure, there was a marked, statistically significant, and reproducible increase in startle amplitude that coincided with presentation of the first CS. This increase, though seemingly triggered by the first CS presentation, persisted beyond its offset and was equally apparent therefore on noise-alone and light–noise trials. An example of this is shown in Fig. 9 for those animals trained with 0.6 mA footshocks. A similar effect was noted in AP5-treated rats trained with 0.3 mA footshocks.

Although other possibilities have not been completely ruled out, a plausible hypothesis is that some form of learning related to fear conditioning had survived intra-amygdala NMDA receptor blockade. In subsequent experiments, we have found that the effect also appears to survive bilateral electrolytic lesions of the amygdala. Thus, it is possible that temporally precise fear responses to specific threats (e.g., fear-potentiated startle to the CS) are mediated by the amygdala, whereas sustained anxiety responses that persist beyond the immediate threat are mediated, at least in part, by structures other than the amygdala. We previously

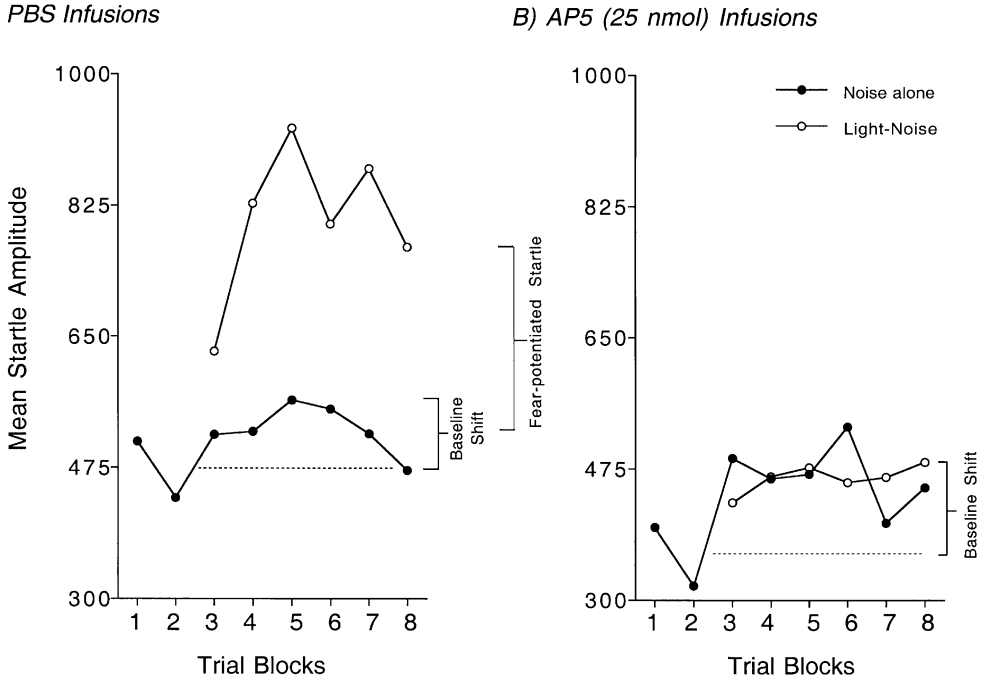

Fig. 9. CS-coincident baseline shift in rats receiving intra-amygdala infusions of D,L-AP5. Rats that received pretraining infusions of PBS showed robust fear-potentiated startle, assessed as the difference in startle amplitude on noise-alone (filled circles) versus intermixed light–noise (open circles) trials, when tested 48 h after training (panel A). These rats also showed an increase in baseline startle that coincided with presentation of the first CS. Rats that received pretraining D,L-AP5 infusions (panel B) did not show fear-potentiated startle, but did show the CS-coincident baseline shift. The data shown above are taken from rats trained with 0.6 mA footshocks. The same pattern was observed in rats trained with 0.3 mA footshocks and also in rats with large electrolytic lesions of the amygdala (data not shown).

reported that several other types of sustained fear/anxiety responses involve the bed nucleus of the stria terminalis (BNST)—a structure with anatomical connections and a neurochemical makeup very similar to the central nucleus of the amygdala (Davis et al., 1997). In particular, pharmacological inactivation of the BNST with the AMPA receptor antagonist, NBQX, disrupts the increase in startle produced by sustained exposure to bright light (Walker and Davis, 2000); electrolytic lesions of the BNST disrupt the increase in startle that develops over days in response to daily footshock exposure (Gewirtz et al., 1998); and axon-sparing lesions of the BNST abolish the increase in startle produced by intracerebroventricular administration of the stress-related peptide, corticotropin releasing hormone (CRH), as do infusions directly into the BNST of the CRH antagonist α-helical CRH (Lee and Davis, 1997). Based on these and related findings, we have speculated that the CS-triggered baseline shift might also be a BNST-dependent phenomenon. However, this hypothesis remains to be tested.

7. Involvement of AMPA receptors in the basolateral and central nucleus of the amygdala in fear learning

Because the basolateral complex is a primary site of sensory convergence within the amygdala (Aggleton and Mishkin, 1986), we wondered whether this subdivision might play a more prominent role in fear learning than does the central nucleus of the amygdala. To assess the relative contribution of each, we recently evaluated the effect on fear learning of pretraining infusions of the AMPA receptor antagonist, NBQX, into the basolateral amygdala and also the central nucleus of the amygdala (AMPA receptors are distributed relatively evenly across both areas; Rainbow et al., 1984). To our surprise, pretraining infusions of NBQX into either area significantly disrupted fear learning (Fig. 10), suggesting that both areas play a role in conditioning.

Although it is difficult to completely rule out the possibility that infusions into the central nucleus disrupted fear learning by diffusing to the basolateral amygdala, we believe this is unlikely. In an earlier study using the same dose (3 μg/side), infusion volume (0.3 μl), infusion rate (0.1 μl/min), and stereotaxic coordinates, we were able to demonstrate differential effects of infusions into the basolateral versus central nucleus infusions on light-enhanced startle (Walker and Davis, 1997b)—an anxiety paradigm in which sustained exposure to bright light elevates startle amplitude (Walker and Davis, 1997a). In that experiment, NBQX infusions into the basolateral amygdala but not the central nucleus of the amygdala disrupted light-enhanced startle. Also, Fanselow and Kim (1994) were able to disrupt fear learning (assessed in a conditioned freezing paradigm) with basolateral but not with central nucleus AP5 infusions, presumably because NMDA receptors are more highly concentrated within the basolateral compared to the central nucleus of the amygdala (Monaghan and Cotman, 1985).

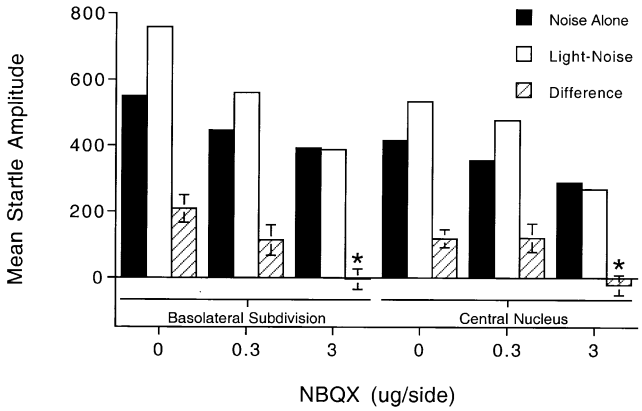

Fig. 10. Pretraining infusions of NBQX into either the basolateral subdivision of the amygdala or the central nucleus of the amygdala disrupt fear learning as assessed with fear-potentiated startle. Previous findings from our laboratory suggest that the infusion parameters used in this experiment allowed for anatomically restricted effects of NBQX. Thus, these data suggest that the basolateral amygdala as well as the central nucleus of the amygdala participate in fear learning. Graphs show the mean startle amplitude on noise-alone and light–noise trials, and the difference in startle amplitude between these two trial types (striped bar ± S.E.M.). Asterisks indicate statistical significance ($P < .05$) versus vehicle controls.

Our results are consistent with those of Wilensky et al. (2000b) who infused the GABA agonist, muscimol, into either the lateral nucleus (a component of the basolateral complex) or the central nucleus of the amygdala prior to training. Infusions into either site produced a disruption of fear learning as assessed with conditioned freezing.

Taken together, these findings suggest that both areas participate in fear learning. As one possibility, both areas may be modified by fear learning and these modifications may be essential for the subsequent expression of fear-potentiated startle. It is also possible that the central nucleus of the amygdala is not modified by fear learning but, instead, influences other areas that in turn influence fear learning. For example, central nucleus activation during training may influence learning via outputs to neuromodulatory centers such as the nucleus basalis of Meynert, the locus coeruleus, the ventral tegmental area, or the dorsolateral tegmental nucleus. These areas, in turn, could directly modulate plasticity via feedback projections to the amygdala, or indirectly modulate plasticity via projections to areas that process CS and US information (for central nucleus modulation of nucleus basalis function and its influence on sensory processing, see Kapp et al., 1992, 1994; for ventral tegmental modulation of amygdala function as it pertains to the expression of fear-potentiated startle, see Borowski and Kokkinidis, 1996; Lamont and Kokkinidis, 1998).

8. Involvement of amygdala NMDA receptors in the extinction of conditioned fear

After conditioning, the predictive power of a given cue can be degraded by repeatedly presenting the cue in the

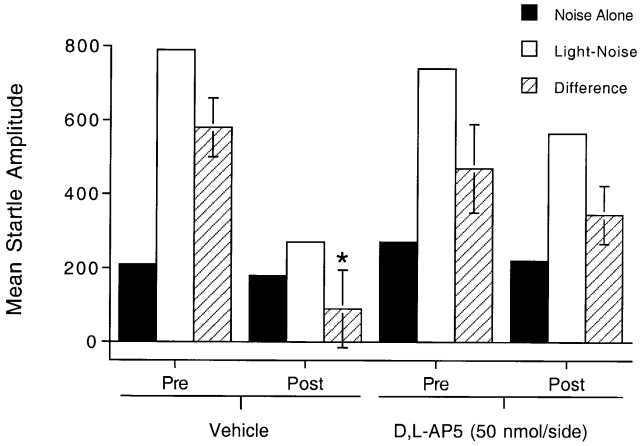

Fig. 11. AP5 blocks extinction when infused into the basolateral nucleus of the amygdala prior to nonreinforced CS presentations. In this experiment, rats received light–shock pairings and the preextinction level of fear-potentiated startle was determined (pre). Rats later received intra-amygdala infusions of either AP5 or vehicle. Immediately thereafter, rats were exposed to a series of nonreinforced light presentations (30 presentations on each of 2 days). 24 h later, rats were retested (post). Rats that received vehicle prior to extinction training showed a significant reduction in fear-potentiated startle; rats that received AP5 did not. Figures show the mean startle amplitude on noise-alone and light–noise trials, along with the difference in startle amplitude between these two trial types (striped bar ± S.E.M.). Asterisk indicates statistical significance ($P < .05$) versus the preextinction baseline.

absence of the US (e.g., in the absence of footshock). As a result, the frequency or amplitude of the conditioned response declines. For example, the ability of a CS to potentiate startle gradually diminishes. This process, termed extinction, is thought by many to reflect new learning rather than unlearning because the previously conditioned response can rapidly be restored if, for example, the animal is given a reminder (e.g., a shock) of the original training (for review, see Davis et al., 2000).

To examine the role of amygdala NMDA receptors in the extinction of conditioned fear, Falls et al. (1992) trained rats with light–shock pairings and subsequently attempted to extinguish the conditioned response by exposing rats on two consecutive days to a series of 30 light presentations without shock. Half of the rats received extinction training following intra-amygdala infusions of D,L-AP5; the other half received extinction training after ACSF infusions. D,L-AP5, at doses of 12.5, 25, and 50 nmol/side, completely blocked extinction that was otherwise robust in ACSF-infused controls (Fig. 11).

These findings are consistent with the view that fear acquisition and fear extinction are forms of learning with at least partially overlapping neuroanatomical substrates and pharmacologies. These findings also point to new strategies for treating clinical anxiety. Could NMDA receptor agonists be used to facilitate the extinction of maladaptive fear and anxiety? Although the therapeutic utility of competitive NMDA receptor agonists might be mitigated by toxic effects of excessive NMDA receptor stimulation, it may be possible to minimize these problems by using partial agonists of NMDA receptor function. One such compound is D-cycloserine, a partial agonist at the glycine binding site on the NMDA receptor complex.

Recently, Walker et al. (submitted) examined the ability of D-cycloserine to facilitate the extinction of conditioned fear in rats that had previously received light–shock pairings. Initial parametric studies indicated that 30 nonreinforced light presentations resulted in a minimally extinguished fear response against which the effects of D-cycloserine could be evaluated. Using this protocol, animals that received systemic infusions of D-cycloserine showed a significant and dose-dependent facilitation of extinction (Fig. 12A) compared to vehicle controls. D-Cycloserine did not increase fear-potentiated startle in animals that did

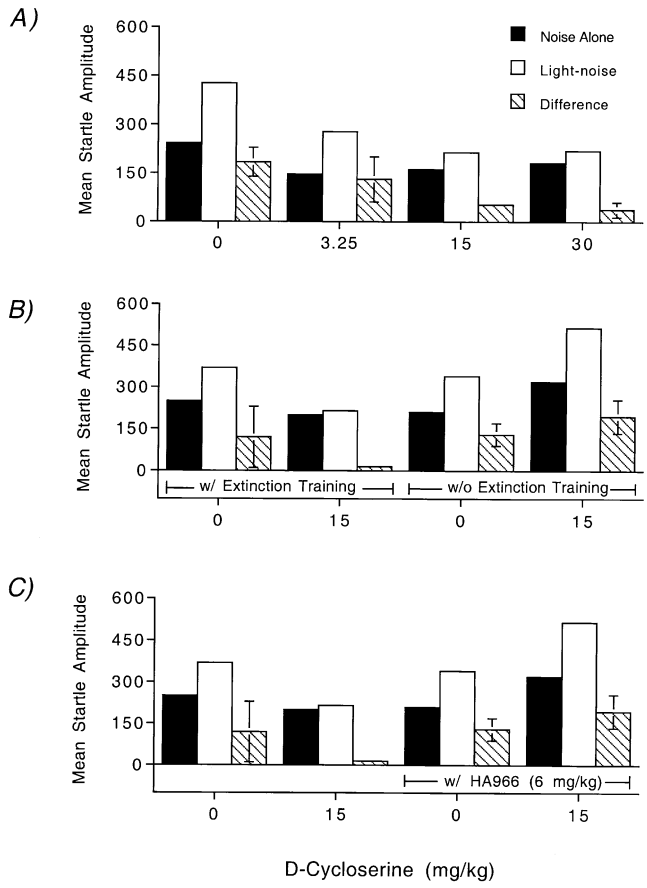

Fig. 12. (Panel A) Systemic administration of D-cycloserine (a partial agonist of the strychnine-insentive glycine binding site on the NMDA receptor), just prior to 30 nonreinforced CS presentations, facilitates extinction in a dose-dependent manner. The data shown above are from the postextinction test. (Panel B) D-Cycloserine does not influence fear-potentiated startle in animals that do not receive extinction training (these animals received context exposure but did not receive nonreinforced CS presentations). (Panel C) The effects of D-cycloserine were blocked by coadministration of the NMDA-associated glycine site antagonist, HA966. Figures show the mean startle amplitude on noise-alone and light–noise trials, along with the difference in startle amplitude between these two trial types (striped bar ± S.E.M.). Asterisk indicates statistical significance ($P < .05$) versus the vehicle control.

not receive extinction training (Fig. 12B), and the effects of D-cycloserine were completely blocked by the glycine modulatory site antagonist, HA966 (Fig. 12C). The primary findings of this study have now been replicated using direct infusions of D-cycloserine into the basolateral amygdala complex. These findings suggest that NMDA receptor agonists might indeed be useful in the treatment of certain types of anxiety disorders (i.e., those with a learning component).

9. Conclusion

Using fear-potentiated startle as a behavioral assay, we have been able to investigate in some detail the neurobiological substrates of fear and fear learning. Although the pharmacological underpinnings of fear conditioning and fear-potentiated startle are complex and appear to reflect the orchestrated interaction of numerous transmitter systems in various brain areas (Davis, 1993; Davis et al., 1993), the data reviewed here suggest a particularly important role of glutamate receptors within the amygdala. For fear conditioning, NMDA receptors within the amygdala play a critical role both in the initial acquisition of fear memories and also in their extinction. AMPA receptors, both in the basolateral amygdala and the central nucleus of the amygdala, appear also to play a necessary role in fear learning. For the expression of fear, amygdala AMPA but not NMDA receptors appear critical. More recent findings have implicated Group II metabotropic glutamate receptors also, both in fear conditioning and in fear expression. By understanding the endogenous neurochemical systems that mediate and regulate fear, it may be possible to develop more effective pharmacological approaches to treating clinical anxiety disorders.

Acknowledgments

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