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# Expression of fear-conditioning is accompanied by increased paired-pulse depression within the amygdala

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**Abstract**

Fear-conditioning is a model of fear learning and anxiety. The lateral nucleus of the amygdala (LA) provides a critical link for relaying thalamic and cortical auditory information to the rest of the amygdala during the fear conditioning process. Alterations in excitatory synaptic transmission in the thalamic to LA pyramidal cells was studied using whole-cell patch clamp recordings in brain slices from fear-conditioned animals. Following paired stimulation of the thalamic afferents, paired-pulse depression (PPD) could be recorded at 200-ms to 2-s intervals. Increasing transmitter release by decreasing the $Mg^{2+}/Ca^{2+}$ ratio enhanced PPD suggesting that PPD is reflective of changes in release probability. Analysis of the pairs of composite, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptor-mediated excitatory postsynaptic currents (EPSCs) showed that there was no correlation between EPSC pairs suggesting that PPD may be mediated through a release-independent mechanism of presynaptic origin. However, AMPA and NMDA receptor mediated PPD had a different time course and magnitude suggesting postsynaptic factors may be involved in PPD. After fear-conditioning PPD of the composite and AMPA receptor-mediated EPSCs was enhanced suggesting that neurotransmitter release may be increased in learned fear. The NMDA receptor-mediated PPD was however not altered in fear-conditioned animals. The differences in response of AMPA and NMDA receptor-mediated PPD suggest that postsynaptic mechanisms may also be involved in the expression of fear conditioning. 

*Keywords:* Fear-conditioning; Learning; Memory; Plasticity; Fear; PPD; PPF; Short-term plasticity; Anxiety; Phobia; EPSC; Emotion; AMPA; NMDA

## 1. Introduction

Fear-conditioning models the attachment of emotional significance to sensory stimuli and provides a means of studying emotional learning in experimental animals. Fear-conditioning is a paradigm in which an originally neutral stimulus called the conditioned stimulus (CS, tone), after pairing with an unconditioned stimulus (UCS, foot shock), can alone then produce a fear response identical to that produced by the UCS. The similarity of the fear learning process in animals and humans is supported by the efficacy of the same experimental paradigms, e.g., potentiated acoustic startle (Grillon et al., 1991; Hamm et al., 1993; Morgan et al., 1996), in eliciting and measuring fear.

One means of measuring fear conditioning is the fear-potentiated startle paradigm. The acoustic startle reflex is a simple, short-latency reflex in which a loud burst of noise elicits a startle response (Davis et al., 1993). The amplitude of the acoustic startle reflex can be potentiated by eliciting an auditory tone in the presence of the CS (e.g., a tone which was previously paired with foot shock) (Brown et al., 1951); this phenomenon is referred to as fear-potentiated startle. The potentiation of the startle response by conditioned fear is thought to be mediated by the amygdala (Davis et al., 1993).

Synaptic depression is a form of short-term plasticity that has been used to analyze release properties of synapses. Paired-pulse depression (PPD) is a phenomenon in which the response to the second of two equal stimuli is smaller than the response to the first. The majority of reports of PPD have investigated the depression of inhibitory transmission (Otis and Mody, 1992; Sugita et al., 1992; Mott et al., 1993; Kang et al., 1994). However, analyses of PPD have been

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performed at excitatory glutamatergic synapses (Mori et al., 1994; Kang, 1995; Thomson and Bannister, 1999; Mennerick and Zorumski, 1995; 1996). Huang and Gean (1994) have demonstrated that PPD of the NMDA component of transmission is present in the amygdala.

PPD is believed to be mediated by presynaptic factors (Davies et al., 1990; Davies and Collingridge, 1993; Mennerick and Zorumski, 1995; Debanne et al., 1996; Thomson, 2000; Waldeck et al., 2000; Oleskevich et al., 2000) although postsynaptic factors may also contribute (Rozov and Burnashev, 1999). Like paired-pulse facilitation (PPF), PPD can be used as a tool to study synaptic transmission. PPD increases with increased neurotransmitter release (Mennerick and Zorumski, 1995; Debanne et al., 1996; Waldeck et al., 2000; Oleskevich et al., 2000). It has been shown in our laboratory that the thalamic-LA synapse exhibits PPF at interstimulus intervals (ISI) of 35–150 ms and that PPF is reduced in fear conditioning (McKernan and Shinnick-Gallagher, 1997). We now report that this same synapse shows PPD at longer ISI and that PPD is enhanced in fear conditioning.

## 2. Materials and methods

### 2.1. Fear conditioning

Male Sprague–Dawley rats (80–120 g) are given water and food ad libitum and maintained on a 12-h light/dark cycle. Fear conditioning is measured using the potentiated startle paradigm adapted from Cassella and Davis (1986) and San Diego Instruments system (San Diego, CA). The rat is placed in the stabilimiter device, in which cage movement results in the displacement of an accelerometer located beneath the stabilimeter (San Diego Instruments). Startle amplitude is defined as a peak accelerometer voltage within 200 ms after startle stimulus onset. In our paradigm, the baseline startle is measured initially in response to a 50-ms white noise burst. The conditioned auditory stimulus (3.7 s of 70 dB white noise tone) is paired with an UCS (0.5 mA foot shock of 0.5 s duration) 10 times a day for 2 days. On the third day, the rats are tested using 30 bursts of white noise (50 ms), 10 of which are preceded by the CS (3.7 s of 70 dB tone). Potentiated startle is defined as a greater than 30% increase over baseline startle amplitude. In the unpaired control group, the conditioned and unconditioned stimuli are applied independently 10 times a day for 2 days and tested in the same paradigm as the paired fear-conditioned animals. Rat brain slices were prepared 48 h after behavioral conditioning and 24 h after the behavioral testing occurred. Previous electrophysiological experiments with fear conditioning (McKernan and Shinnick-Gallagher, 1997) were performed blind with respect to the behavioral treatment but no significant difference was measured between blinded and nonblinded experiments and the results were pooled. In the present experiments, both blinded and nonblinded experiments were pooled.

### 2.2. Preparation of brain slices

Rats were rapidly decapitated and the brain quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) bubbled with a mixture of 95% $O_2$ and 5% $CO_2$. Slices of 450 μm are prepared and kept at room temperature for 1 h, then transferred to the recording chamber where they are incubated at $33 \pm 1$ °C for 40 min before recording. The slices are perfused with oxygenated ACSF (pH ≈ 7.4) at 1.5 ml/min (chamber volume). The composition of the ACSF is as follows (mM): NaCl, 117; KCl, 3; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 25; $NaH_2PO_4$, 1.2; glucose, 11.5. Magnesium was omitted in $Mg^{2+}$-free ACSF.

### 2.3. Whole-cell recording

Voltage clamp recordings in the whole cell configuration are used as described by Blanton et al. (1989). Patch electrodes (3–5 MΩ) are pulled with a Flaming–Brown Model P80 puller (Sutter instruments, Novato, CA) from a glass capillary (1.13 mm I.D., 1.5 mm O.D.) and filled with internal

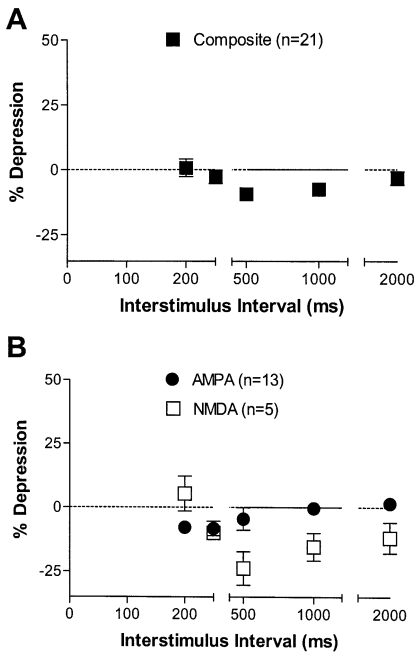


Fig. 1. PPD at the thalamic-LA synapse. (A) Mean depression is plotted as a function of interstimulus intervals from 200 to 2000 ms. Depression is maximal at an interval (ISI) of 500 ms and gradually returns to baseline by the 2000 ms ISI ($n = 21$). (B) PPD of the AMPA current vs. PPD of the NMDA current. Pharmacologically separating the components of the composite EPSC reveals differences between PPD of the AMPA and NMDA currents. The AMPA current was isolated by superfusion of 50 μM D-APV, the NMDA current by the superfusion of 5 μM NBQX and $Mg^{2+}$-free solution. Mean depression is plotted as a function of ISI from 200 to 2000 ms. PPD of the NMDA component shows a greater magnitude and a slower time course than PPD of the AMPA component.

solution (mM) as follows: Cs-gluconate, 115; NaCl, 5; EGTA, 1; $CaCl_2$, 0.3; $MgCl_2$, 2; Na-ATP, 5; Na-GTP, 0.4; HEPES, 10. The pH (7.2) and osmolarity (280 mOsm) are adjusted using 1 M CsOH and sucrose respectively. Voltage clamp recordings are performed using an Axoclamp 2 A amplifier (Axon Instruments) and membrane current and voltage signals are low-pass filtered at 1 kHz and digitized (Digidata 1200, Axon Instruments) at 5 kHz for computer storage. Data acquisition and analysis are accomplished using pClamp 7.01 and Clampfit (Axon Instruments). Excitatory postsynaptic current (EPSC) is elicited in pyramidal cells at the lateral amygdala (LA) by applying a square pulse of 150 μs duration and variable intensity (4–10 V) through a bipolar electrode placed on fibers emerging from the internal capsule and projecting to the LA (LeDoux et al., 1985); typically the stimulus intensity used was that needed to elicit an EPSC of 70–80% maximal EPSC amplitude. The peak amplitude for EPSC is measured as the difference between the baseline immediately before each stimulus artifact and the peak of the EPSC. For the paired-pulse stimulation, two stimuli of identical strength are applied in succession at different ISI (200–2000 ms) giving rise to $EPSC_1$ and $EPSC_2$. Paired stimulation is applied at a rate of 0.1 Hz. The percentage of depression is calculated according to the following formula: $[(EPSC_2 - EPSC_1)/EPSC_1]100$.

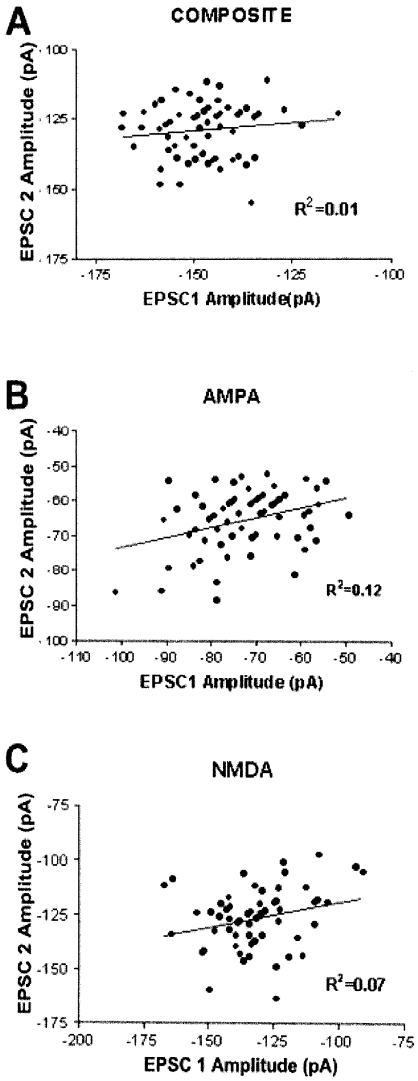


Fig. 2. Lack of correlation of EPSCs suggests PPD is mediated presynaptically. (A) PPD of the composite response is presynaptically mediated. Plot of correlation between EPSC1 and EPSC2 amplitudes. The insignificant correlation ($R^2 = .06$, $n = 7$) between EPSC1 amplitude vs. EPSC2 amplitude suggests that both vary independently. (B) PPD of the AMPA response is presynaptically mediated. Plot of correlation between AMPA EPSC1 and AMPA EPSC2 amplitudes. The insignificant correlation ($R^2 = .08$, $n = 6$) between EPSC1 amplitude vs. EPSC2 amplitude suggests that both vary independently. (C) PPD of the NMDA response is presynaptically mediated. Plot of correlation between NMDA EPSC1 and NMDA EPSC2 amplitudes. The insignificant correlation ($R^2 = .08$, $n = 5$) between NMDA EPSC1 amplitude vs. NMDA EPSC2 amplitude suggests that NMDA current PPD is mediated by presynaptic factors.

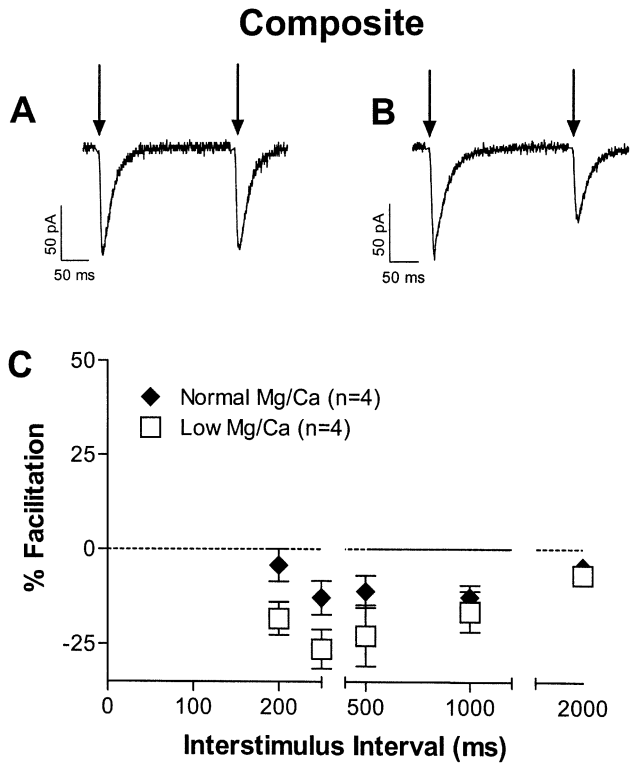


Fig. 3. PPD increases with increased transmitter release. Traces in (A) and (B) represent examples of PPD at a 250-ms ISI at normal (A) and decreased (B) external $[Mg^{2+}]/[Ca^{2+}]$ ratio; traces shown are averages of five recorded traces. $V_{HOLD} = -60$ mV. (C) PPD is significantly increased in lowered external $Mg^{2+}/Ca^{2+}$. Plot illustrates the time course of PPD, with percent depression plotted as a function of ISI.

## 3. Results

### 3.1. Characteristics of PPD at the thalamic-LA synapse

To assess the pharmacological properties of PPD in the thalamic to LA pathway we determined PPD of composite and AMPA and NMDA receptor-mediated EPSCs. The

input–output relationship was assessed with every synaptic potential by varying the input stimulus and measuring the EPSC amplitude. EPSC amplitudes equivalent to 80% of maximum value were used for measuring PPD. These same EPSCs were used for measuring PPF reported earlier (McKernan and Shinnick-Gallagher, 1997). Composite EPSCs elicited at the thalamic-LA synapse exhibited PPD at ISI ranging from 250 to 1000 ms (Fig. 1A). The magnitude of the depression was small; the maximal PPD measured at 500 ms ISI was only $-9.3\% \pm 2.4\%$ ($n=21$). Pharmacologically separating the composite EPSC into AMPA and NMDA EPSCs revealed differences in the time course and magnitude of PPD (Fig. 1B). The AMPA receptor mediated PPD, isolated in 50 μM D-2-amino-5-phosphonovaleric acid (D-APV), displayed maximal depression ($-8.2\% \pm 2.8\%$) at 250 ms ISI, and returned to baseline by 1 s. On the other hand, the depression of the NMDA receptor-mediated PPD, isolated with 5 μM 6-nitro-7-sulphamoylbenzo[*f*]quinoxaline-2,3-dione (NBQX) and $Mg^{2+}$-free solution, was maximal at 500 ms ISI ($-26.3\% \pm 4.4\%$) and did not recover completely by 2 s.

Although PPD is thought to reflect presynaptic mechanisms (Kang, 1995; Mennerick and Zorumski, 1995; Debanne et al., 1996; Waldeck et al., 2000; Oleskevich et al., 2000), the possibility of postsynaptic involvement in PPD at the thalamic-LA synapse still exists. To investigate this possibility more directly, we examined the correlation between the amplitudes of $EPSC_1$ and $EPSC_2$, the two responses evoked by the pair of pulses. A positive correlation between the two amplitudes suggests a release-dependent process of pre-or postsynaptic origin, while the lack of a correlation suggests a release-independent process of presynaptic origin (Lin and Faber, 1988).

We determined the correlation between $EPSC_1$ and $EPSC_2$ at the 250 ms ISI for the composite EPSC as well as the isolated AMPA and NMDA components (Fig. 2). There was no positive correlation between $EPSC_1$ and $EPSC_2$ for the composite response ($R^2=.06 \pm .02$, $n=7$; Fig. 2A), the isolated AMPA ($R^2=.08 \pm .02$, $n=6$; Fig. 2B) or NMDA ($R^2=.08 \pm .01$, $n=5$; Fig. 2C) receptor-mediated components. These data provide supportive evidence that presynaptic factors play a significant role in PPD at this synapse.

### *3.2. PPD increases with increased transmitter release probability*

PPD has been shown to increase with manipulations that increase transmitter release, such as lowering the external $Mg^{2+}/Ca^{2+}$ ratio (Mennerick and Zorumski, 1995; Debanne et al., 1996; Waldeck et al., 2000; Oleskevich et al., 2000). We investigated the effect of lowering the external $Mg^{2+}/Ca^{2+}$ ratio from 0.48 to 0.16 (control: $Mg^{2+}$: 1.2 mM, $Ca^{2+}$:

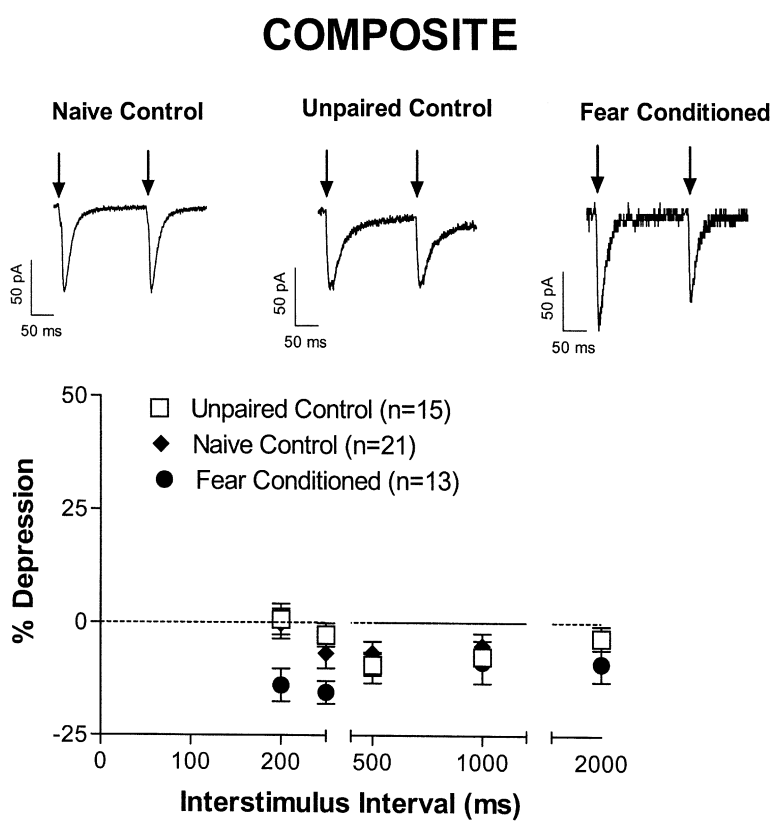


Fig. 4. PPD of the composite EPSC is increased in fear conditioned animals. Traces on top represent examples of PPD of composite EPSCs at 200-ms ISI for the three groups; recordings shown are averages of five traces. $V_{HOLD}=-60$ mV. Plot below illustrates the time course of PPD, with percent depression plotted as a function of ISI. PPD of composite EPSCs is significantly increased in fear-conditioned animals at the 200- and 250-ms ISI.

2.5 mM; test: $Mg^{2+}$: 0.5 mM, $Ca^{2+}$: 3.2 mM) on the magnitude of PPD of the composite EPSC at this synapse (Fig. 3). PPD was increased with the lower external $Mg^{2+}/Ca^{2+}$ ratio; this difference was significant at the 200- ($P<.05$, paired $t$ test) and 250-ms ($P<.01$, paired $t$ test) ISI. These data suggest that increasing transmitter release at this synapse increases PPD.

### 3.3. PPD is increased in fear conditioned animals

Previous studies in this laboratory have shown that synaptic transmission is potentiated in fear conditioned animals (McKernan and Shinnick-Gallagher, 1997). This potentiation was due, at least in part, to an increase in transmitter release, which was reflected in a decrease in the magnitude of PPF between 35 and 200 ms ISI. In this present work, we compared the degree of PPD, which occurs between 250 and 1000 ms ISI, in neurons from three groups of animals: fear-conditioned animals, which were exposed to paired tone and foot shock, unpaired control animals, which were exposed to the tone and foot shock in an unpaired fashion, and naïve control animals.

Fear conditioned animals exhibited greater PPD of the composite EPSC than unpaired or naïve control animals (Fig. 4). This difference was significant at the 200 ms [$F(2,40)=3.90$, $P=.03$, one-way ANOVA; $P<.05$, Newman–Keuls] and 250 ms [$F(2,40)=3.66$, $P=.03$, one-way ANOVA; $P<.05$, Newman–Keuls] ISI. There was no significant difference in PPD of the composite EPSCs in unpaired and naïve control animals.

We further analyzed the contribution of the different classes of glutamate ionotropic receptors to the expression of fear conditioning by examining the magnitude of PPD of the isolated AMPA and NMDA receptor-mediated EPSCs. PPD of the AMPA EPSC is significantly increased in fear-conditioned animals (Fig. 5). This difference was significant at the 200 ms [$F(2,32)=3.49$, $P<.04$, one-way ANOVA; $P<.05$, Newman–Keuls] and 250 ms [$F(2,31)=7.39$, $P=.002$, one-way ANOVA; $P<.01$, Newman–Keuls] ISI. At the 500 ms ISI, the difference was not quite significant [$F(2,21)=2.64$, $P=.09$, one-way ANOVA].

When we compared PPD of the isolated NMDA receptor-mediated EPSC, there was no difference between the naïve control and fear-conditioned animals (Fig. 6). However, there was a difference in NMDA PPD between the unpaired and naive control groups at 500 ms ISI. NMDA PPD in the unpaired control animals differed from the naïve control and fear conditioned animals, both of which showed

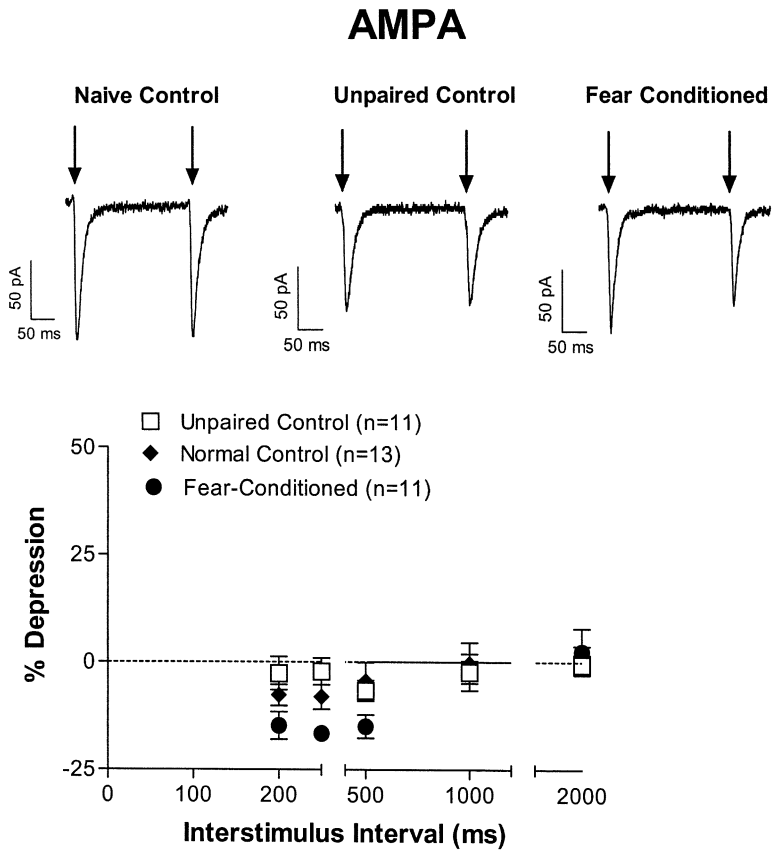


Fig. 5. PPD of the AMPA component is increased in fear-conditioned animals. Traces across the top represent examples of PPD at 250-ms ISI for the three groups; traces shown are averages of five pairs of EPSCs. $V_{HOLD}=-60$ mV. Plot below illustrates the time course of PPD, with percent depression plotted as a function of ISI. PPD of the AMPA current is significantly increased in fear-conditioned animals at the 200- and 250-ms ISI.

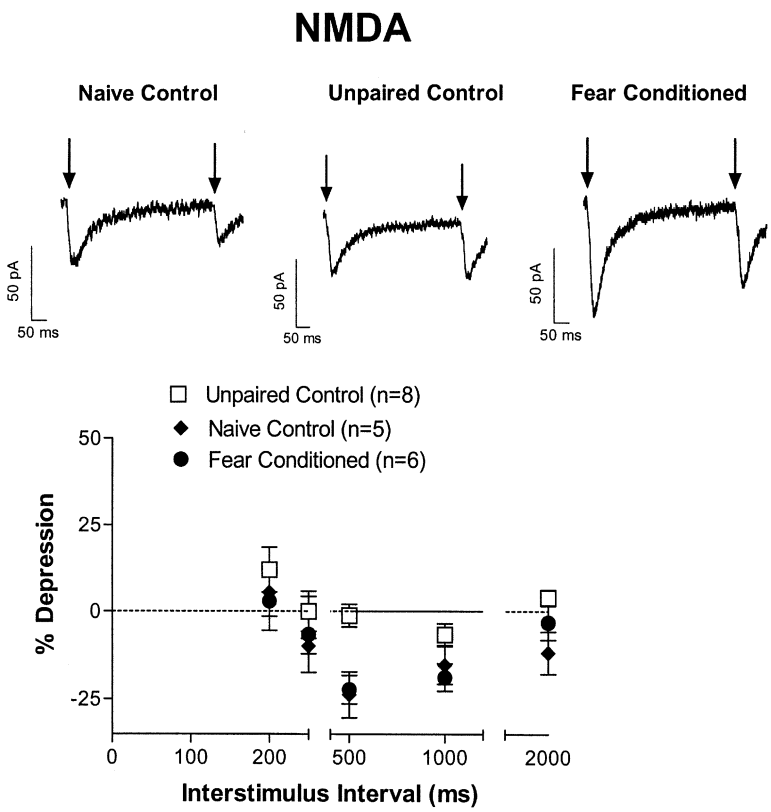


Fig. 6. PPD of the NMDA component is not increased in fear-conditioned animals. Traces across the top represent examples of PPD at 500-ms ISI for the three groups; EPSCs shown are averages of five recorded traces. $V_{HOLD} = -60$ mV. Plot below illustrates the time course of PPD, with percent depression plotted as a function of ISI. PPD is significantly decreased in unpaired control animals but not fear-conditioned animals at 500-ms ISI.

similar PPD. The degree of PPD in unpaired control animals was significantly less than that of the other two groups at the 500 ms ISI [$F(2,14) = 13.84$, $P < .001$; $P < .01$, Newman–Keuls; Fig. 6]. These data suggest that, with fear conditioning, the magnitude of PPD mediated through the NMDA receptor was not altered.

## 4. Discussion

The main findings of this study are that at the thalamic-LA synapse: (1) the time course of AMPA and NMDA receptor-mediated PPD are different and a number of its characteristics suggest that PPD at this synapse may be mediated at least in part presynaptically. (2) Fear conditioning increases PPD of the composite EPSC at 200 and 250 ms ISI as compared to naïve or unpaired control animals; and (3) PPD mediated through the AMPA receptor but not the NMDA receptor is increased in fear conditioning.

### *4.1. PPD at the MGN-LA synapse: NMDA and AMPA components*

PPD is evident at the thalamic-LA synapse with ISIs ranging from 200 to 1000 ms. Since PPD is believed to be mediated presynaptically, one would predict that PPD would be expressed similarly at different subtypes of postsynaptic glutamate receptors. However, when the composite EPSC is isolated into its constituent AMPA and NMDA components, differences in the time course and the magnitude of PPD of the two components are apparent. Discrepancies in the magnitude (Mott et al., 1993; Mennerick and Zorumski, 1996) and time course (Kang et al., 1994) of PPD for different postsynaptic receptor subtypes have been reported previously. These differences have been attributed to a segregation of presynaptic inputs relative to the postsynaptic receptor types they contact (Kang, 1995; Kang et al., 1994; Otis and Mody, 1992). Our synapse shows a difference in the degree of depression between AMPA (−8.2%) and NMDA (−26.3%) in naïve control animals. There is also a difference in the ISI at which the maximum PPD is measured for the AMPA (250 ms) and NMDA (500 ms) EPSC pairs. Recent anatomical evidence in the hippocampus suggests that at most CA1 synapses, these receptors may be located together on spines (Racca et al., 2000) suggesting that, if a similar distribution exists in the amygdala, segregated inputs not may explain the differences between AMPA and NMDA PPD. These differences in AMPA and NMDA receptor-mediated PPD then could be due to a number of pre-and/or postsynaptic factors.

### 4.2. Is PPD mediated through a presynaptic mechanism?

The general consensus of numerous investigations is that PPD is mediated by presynaptic factors (Davies and Collingridge, 1993; Davies et al., 1990; Mennerick and Zorumski, 1995; Kang, 1995; Debanne et al., 1996; Thomson, 2000; Waldeck et al., 2000; Oleskevich et al., 2000). Several pieces of evidence in this study support that view at the thalamic-LA synapse. We showed that a decrease in magnesium to calcium ratio, known to increase neurotransmitter release, increased NMDA receptor-mediated PPD. The lack of correlation between $EPSC_1$ and $EPSC_2$ in the composite, and AMPA and NMDA receptor-mediated EPSCs also suggests that PPD is regulated by a release-independent presynaptic process (Lin and Faber, 1988). Since the correlation analysis was carried out only at the 250 ms ISI, it is unclear whether the lack of correlation is consistent at all ISIs.

PPD has been ascribed to two separate presynaptic mechanisms, one, a result of a depletion of immediately releasable stores of transmitter (Zucker, 1989), the other, involving the feedback of released transmitter onto presynaptic receptors (Davies and Collingridge, 1993). The PPF at this synapse at shorter ISI (35–150 ms; McKernan and Shinnick-Gallagher, 1996, 1997) suggests that the first possibility may be unlikely. However, in situations where the initial release probability is high (e.g., lowered $Mg^{2+}/Ca^{2+}$ ratio, fear conditioned animals), transmitter depletion may play a larger role. The second possibility can be incorporated into the model of segregated inputs by the suggestion that different subsets of inputs may be regulated by different types of presynaptic receptors (Kang, 1995). If PPD at this synapse involves presynaptic receptors, the coexistence of PPF and PPD could be explained in several ways. First, the process of binding to presynaptic receptors and affecting a decrease in neurotransmitter release may involve a delay, resulting in the expression of PPD only at longer ISI. Second, PPD may be present at all ISI, but its expression at shorter intervals (35–150 ms) is masked by the transient rise in intracellular $Ca^{2+}$ that results in PPF.

### 4.3. Are postsynaptic factors involved in PPD?

Differences in PPD between the AMPA and NMDA components could be rooted in postsynaptic involvement in PPD. If PPD was controlled solely by presynaptic factors, the time course and magnitude of PPD would be expected to be the same for the AMPA and NMDA current. Nevertheless, differences similar to those reported here have been measured at other synapses. Differences in the magnitude of PPD of the NMDA and non-NMDA components of cortical EPSCs have also been attributed to postsynaptic factors influencing the NMDA current; the degree of PPD was decreased by partial block of NMDA receptors and partial relief of the $Mg^{2+}$ block increased NMDA PPD (Mennerick and Zorumski, 1996). Thus, our isolation of the NMDA current in NBQX and $Mg^{2+}$-free medium may serve to magnify the degree of PPD recorded. Furthermore, Huang and Gean (1994) have shown that NMDA PPD in the amygdala could be due to activation of $GABA_B$ receptors after the first stimulation. Since we did not block GABA receptors, it is possible that presynaptic $GABA_B$ and postsynaptic $GABA_A$may influence the magnitude of PPD we recorded. Postsynaptic $GABA_B$ receptors were blocked by the intracellular cesium in our electrode solution. Finally, feedback onto presynaptic glutamate receptors may affect PPD such that use of the antagonists in our studies may influence findings. Although we did not test this possibility in the present experiments we have considered this situation with respect to PPF at shorter ISI. Several pieces of evidence suggest that this may not be a factor: (1) injecting an NMDA antagonist, MK 801, intracellularly produced the same effect as superfusing APV in the same neuron and (2) the difference between PPF measured by subtraction (as the APV-sensitive EPSC) and that measured in $Mg^{2+}$-free NBQX solution was similar (Zinebi et al., in press).

### 4.4. PPD is increased in fear-conditioned animals

PPD is increased in lateral amygdala neurons from fear-conditioned animals. These data suggest that, if PPD is indeed a measure of transmitter release, glutamate release is increased in fear-conditioned animals. These data correspond well with previous findings in our laboratory showing a decrease in PPF in fear-conditioned animals (McKernan and Shinnick-Gallagher, 1997). Decreases in the degree of PPF are also thought to indicate increases in transmitter release. With both measures (PPF and PPD), the changes are evident in the composite and isolated AMPA response of fear conditioned animals.

No increase in PPD of the NMDA receptor-mediated EPSC was observed in fear-conditioned animals. These data are similar to those measured in fear-conditioned animals with NMDA receptor-mediated PPF (Zinebi et al., 2000). With NMDA receptor-mediated PPD, the only difference recorded between the experimental groups occurs at the 500-ms ISI where unpaired control animals show significantly less PPD than naïve control or fear conditioned animals. The reason for selective changes in the PPD of the NMDA component of unpaired control animals is unclear. Unpaired controls are exposed to the tone (CS) and foot shock (UCS) in an unpaired manner, but they may undergo some contextual conditioning to the extent that the foot shock occurs in the experimental chamber. The expression of contextual, but not discrete cue, fear conditioning has been shown to involve NMDA receptors (Maren, 1996). This change in unpaired control animals may reflect unique contextually induced alterations in NMDA receptors.

In summary, the present study shows that in the expression of learned fear, PPD of composite and AMPA receptor mediated EPSC is enhanced. The weight of the evidence suggests that PPD may be mediated primarily

through presynaptic factors suggesting that in the expression of fear conditioning glutamate release is enhanced. However, NMDA receptor-mediated PPD is not altered in fear conditioning. This difference in the response to NMDA and AMPA receptor-mediated PPD suggests that postsynaptic factors may also be involved in the expression of learned fear.

## Acknowledgments

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