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Behavioral and neurophysiological evidence that lateral paracapsular GABAergic synapses in the basolateral amygdala contribute to the acquisition and extinction of fear learning

MJ Skelly, AM Chappell, OJ Ariwodola, and JL Weiner

Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157

Abstract

The lateral/basolateral amygdala (BLA) is crucial to the acquisition and extinction of Pavlovian fear conditioning, and synaptic plasticity in this region is considered to be a neural correlate of learned fear. We recently reported that activation of BLA β -adrenoreceptors (β -ARs) selectively enhances lateral paracapsular (LPC) feed-forward GABAergic inhibition onto BLA pyramidal neurons, and that intra-BLA infusion of a β -AR agonist reduces measures of unconditioned anxiety-like behavior. Here, we utilized a combination of behavioral and electrophysiological approaches to characterize the role of BLA LPCs in the acquisition of fear and extinction learning in adult male Long-Evans rats. We report that intra-BLA microinjection of β -AR agonists (BRL37344 or SR58611A, 1 μ g/0.5 μ l/side) prior to training fear conditioning or extinction blocks the expression of these behaviors 24 hours later. Furthermore, *ex vivo* low-frequency stimulation of the external capsule (LFS; 1 Hz, 15 min), which engages LPC synapses, induces LTP of BLA fEPSPs, while application of a β -AR agonist (SR58611A, 5 μ M) induces LTD of fEPSPs when combined with LFS. Interestingly, fEPSP LTP is not observed in recordings from fear conditioned animals, suggesting that fear learning may engage the same mechanisms that induce synaptic plasticity at this input. In support of this, we find that LFS produces LTD of inhibitory postsynaptic currents (iLTD) at LPC GABAergic synapses, and that this effect is also absent following fear conditioning. Taken together, these data provide preliminary evidence that modulation of LPC GABAergic synapses can influence the acquisition and extinction of fear learning and related synaptic plasticity in the BLA.

Keywords

Fear-Potentiated Startle; Inhibitory Plasticity; Noradrenaline

Corresponding Author: Jeff L. Weiner, Ph.D., Professor, Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, Tel: (336) 716-8692, Fax: (336) 716-8501, jweiner@wakehealth.edu.

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1. Introduction

Disruptions of fear and extinction learning are symptomatic of many psychopathologies, such as post-traumatic stress disorder (PTSD) (Maren et al., 2013), and elucidating the neural substrates of non-pathological fear learning may assist in identifying the maladaptations underlying PTSD and related disorders. Pavlovian fear conditioning is a well-established behavioral paradigm commonly used to study the neurobiology of fear memory formation, and efforts to date have revealed that the basolateral amygdala (BLA) plays an important role in the acquisition, expression, and extinction of conditioned fear (Maren, 2005; Likhtik and Paz, 2015). During fear conditioning, sensory afferents encoding the paired presentation of a conditioned stimulus (CS) and aversive unconditioned stimulus (US) converge on BLA pyramidal projection neurons (Maren, 2005), resulting in long-term potentiation (LTP) of CS inputs (Pape and Pare, 2010). This plasticity increases CS-evoked pyramidal neuron excitability, and is considered a synaptic correlate of fear memory (Walker and Davis, 2002; Maren, 2005).

BLA excitability is tightly regulated by two distinct populations of GABAergic interneurons; local inhibitory interneurons (Rainnie et al., 2006; Woodruff and Sah, 2007; Jasnow et al., 2009), and lateral paracapsular cells (LPCs) (Marowsky et al., 2005; Silberman et al., 2010). While GABAergic inhibition from these inputs typically constrains the induction of LTP at excitatory afferent synapses, this brake may be lifted during fear conditioning (Ehrlich et al., 2009; Lee et al., 2013). In support of this, plasticity at local inhibitory synapses has been implicated in the expression and extinction of learned fear (Marsicano et al., 2002; Azad et al., 2004; Szinyei et al., 2007). Similarly, there is some evidence that suppression of LPC inhibitory inputs onto BLA pyramidal cells likewise contributes to fear memory formation (Bissiere et al., 2003; Manzanares et al., 2005), although this relationship has yet to be directly assessed.

The LPCs are well positioned to modulate fear and extinction learning, as they are activated by thalamic and cortical afferents conveying sensory information to the BLA (Marowsky et al., 2005; Strobel et al., 2015), and excitatory plasticity at these cortical inputs contributes to fear learning (Tsvetkov et al., 2002; Schroeder and Shinnick-Gallagher, 2005). We recently demonstrated that activation of β 3-adrenoreceptors (β 3-ARs) selectively enhances LPC feed-forward GABAergic inhibition onto BLA pyramidal cells, and that potentiating this inhibitory input via targeted delivery of β 3-AR pharmacological agents disrupts amygdala-dependent behaviors (Silberman et al., 2010; Butler et al., 2014). Thus, as β 3-ARs can be used to selectively enhance LPC synapses *in vivo*, we hypothesized that pharmacologically enhancing inhibitory signaling at these synapses prior to CS/US pairing might similarly disrupt fear and extinction learning *in vivo* and BLA synaptic plasticity *ex vivo*. We further hypothesized that fear learning would promote long term depression at LPC synapses (iLTD), leading to an occlusion of electrically-evoked plasticity.

2. Materials and Methods

2.1 Subjects

Adult male Long-Evans rats (Harlan Laboratories, Indianapolis, IN, USA) arrived weighing 350g (appx. 12 weeks old) and maintained on a 12-h light/dark cycle (lights on 7:00 A.M. to 7:00 P.M.) with ad libitum access to food and water. Animals were singly housed for at least one week but no longer than three weeks prior to experimentation. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Wake Forest University School of Medicine Animal Care and Use Committee.

2.2 Behavioral Experiments

Because we have found that pair housing adult male Long-Evans rats following intracranial cannulation surgeries increases the incidence of injury and infection, all animals used in these studies were singly housed throughout. Following one week of acclimation to the housing environment, surgery animals were implanted with indwelling stainless steel guide cannulae bilaterally above the BLA, as previously described (Butler et al., 2014). Following recovery, rats received an intra-BLA vehicle injection to acclimate them to the microinfusion procedure. Forty-eight hours later, two separate cohorts of animals received microinjections of either a β 3-AR agonist (BRL37344 or SR58611A, 1 μ g/0.5 μ l/side) or vehicle (0.9% NaCl) into the BLA immediately prior to fear conditioning. Drug solutions were prepared fresh on each microinjection day.

During fear conditioning, animals were placed in individual plexiglass tubes (10" (L) $\times$ 5" (ID)) containing a grid floor equipped to convey footshocks and enclosed in sound-attenuated startle chambers (San Diego Instruments, San Diego, CA, USA). Following a five minute acclimation, a light conditioned stimulus (CS) was presented (duration 2 sec) with a 0.5 mA co-terminating foot shock (duration 0.5 sec). This combination was repeated 10 times, with each pairing separated by a two-minute inter-stimulus interval.

Fear learning was tested 24 hours later using the fear-potentiated startle paradigm, during which startle-inducing noise bursts (95 db) were presented either alone (10 presentations) or paired with the CS (10 presentations) in a pseudo-random order. Response magnitude was assessed using a stabilimeter/accelerometer device; movement in response to each noise burst displaced the accelerometer and the magnitude of this displacement was converted to a voltage. Fear-potentiated startle was assessed by comparing the magnitude of accelerometer displacement in response to the noise alone versus the noise presented in the presence of the CS.

A third cohort of animals was fear conditioned as described above, except that no microinjections were delivered to these animals prior to fear training. Twenty-four hours after testing, these animals received an intra-BLA microinjection of BRL37344 (1 μ g/0.5 μ l/side) or saline vehicle (0.9% NaCl) immediately prior to extinction training. During extinction, animals were returned to the experimental chambers and exposed to 30 consecutive presentations of the CS alone. Twenty-four hours after extinction training, extinction learning was assessed using the fear-potentiated startle procedure.

2.3 Electrophysiological Experiments

Animals used for electrophysiological experiments were fear conditioned and tested for confirmation of fear memory formation twenty-four hours later, as described above. Animals were sacrificed and slices were taken one hour after the testing session; naïve animals were taken directly from their home cage prior to being sacrificed. Transverse amygdala slices (400 μm) were prepared and maintained as described elsewhere (Rau et al., 2014). One to two cells/field excitatory post-synaptic potentials (fEPSPs) were recorded from each animal. Recording electrodes for all field and patch clamp experiments were prepared from filamented borosilicate glass capillary tubes (inner diameter, 0.86 mm) using a horizontal micropipette puller (P-97; Sutter Instruments, Novato, CA). During patch clamp experiments, GABA_A receptor-mediated inhibitory post-synaptic currents (eIPSCs) were recorded using an internal solution containing 130 mM K-gluconate, 10 mM KCl, 1 mM EGTA, 100 μM CaCl₂, 2 mM Mg-ATP, 200 μM Tris-guanosine, 5'-triphosphate, and 10 mM HEPES; internal pH was adjusted to 7.2 with KOH, 275–280 mOsm. fEPSP recordings were obtained using a 0.9% NaCl internal solution. For patch clamp experiments, 5 mM N-(2,6-dimethyl-phenylcarbamoylmethyl)-triethylammonium chloride (QX-314) was included in the recording solution to block voltage-gated sodium currents and GABA_B IPSCs in the BLA neurons being recorded. Whole-cell patch-clamp recordings were conducted from BLA pyramidal neurons voltage-clamped at -40 mV. Only cells with a stable access resistance of 5–20 M Ω were used in these experiments. Whole-cell currents were acquired using an Axoclamp 2B or Axopatch 200B amplifier, digitized (Digidata1200 or Digidata 1321A; Axon Instruments, Union City, CA), and analyzed offline using an IBM-compatible personal computer and pClamp 9.0 software (Axon Instruments).

eIPSCs were pharmacologically isolated using a mixture of 50 μM APV and 20 μM DNQX to block NMDA and AMPA/kainate receptors, respectively. Drug stock solutions were prepared in advance, aliquoted, and frozen until use. No GABA or glutamate receptor blockers were applied to slices during fEPSP experiments; in the SR58611A condition, the drug was applied prior to recording baseline and was continually applied throughout the experiment. There was no significant effect of this β 3-AR agonist on fEPSP amplitude (data not shown). Synaptic currents and fEPSCs were evoked every 20 s by electrical stimulation (0.2 ms duration) using a concentric bipolar stimulating electrode (FHC, Bowdoinham, ME) placed along the external capsule to target LPCs/cortical projection neurons. Recording electrodes were aimed at the basal portion of the BLA. Synaptic plasticity was induced by applying a low-frequency stimulation protocol (LFS; 1 Hz, 15 min). All recordings were performed at room temperature (22–25°C), as we have shown that this temperature promotes patch stability without altering the pharmacological properties of GABA_A IPSCs (Ariwodola and Weiner, 2004).

2.4 Statistics

Post-LFS change in the amplitude of eIPSCs and fEPSPs were analyzed using separate two-way repeated measures ANOVAs comparing the average percent change from baseline of fEPSP or eIPSC amplitude as a function of time and experimental condition. Additionally, the average percent change in eIPSC amplitude following LFS was analyzed using an unpaired t-test, while change in fEPSP amplitude was analyzed using a one-way ANOVA

with post-hoc pairwise multiple comparisons (Holm-Sidak method). Within group differences in average startle amplitude (noise alone versus CS-paired noise) were analyzed using paired t-tests, and between group differences in the percent change in startle amplitude (CS-paired noise/noise alone) were analyzed using unpaired t-tests. When data were not normally distributed, Wilcoxon signed ranks tests were used in the place of paired t-tests, and Mann-Whitney Rank Sum tests were used in place of unpaired t-tests. The alpha level for all analyses was set at $p < 0.05$.

3. Results

Enhancement of LPC synapses via microinjection of the $\beta 3$ -AR agonist BRL37344 (1 $\mu\text{g}/0.5 \mu\text{L}/\text{side}$) bilaterally into the BLA previous to training fear conditioning blocked the expression of fear learning 24 hrs later (Fig. 1). Specifically, the average startle response elicited by CS-paired noise bursts was not significantly increased relative to the average noisealone response (Fig. 1a; $n = 8$, $t(7) = 1.376$, $p = 0.211$). In contrast, vehicle-treated animals exhibited a significantly potentiated startle response to the CS-paired noise (Fig. 1a; $n = 8$, $z = 2.521$, $p = 0.014$). Accordingly, the percent change in startle amplitude was greater among vehicle-treated animals than BRL37344-treated animals (Fig. 1b; $U = 11$, $p = 0.031$). Similarly, selective potentiation of LPC synapses via intra-BLA microinjection of the $\beta 3$ -AR agonist SR58611A (1 $\mu\text{g}/0.5 \mu\text{L}/\text{side}$) blocked the fear-potentiated startle response (Fig. 1c; $n = 8$, $t(7) = 1.395$, $p = 0.206$), which remained unaffected in vehicle-treated controls (Fig. 1c; $n = 8$, $t(7) = 3.341$, $p = 0.012$). The percent change in startle response was likewise significantly increased in vehicle-treated animals relative to SR58611A-treated animals (Fig. 1d; $t(14) = 2.608$, $p = 0.021$).

Enhancement of LPC inhibitory synapses with bilateral intra-BLA microinjection of BRL37344 (1 $\mu\text{g}/0.5 \mu\text{L}/\text{side}$) before training extinction also blocked the expression of extinction learning 24 hrs later (Fig. 2). Animals in both groups acquired fear conditioning normally, as evidenced by exhibiting increased startle responses, on average, to CS-paired noise burst (Fig. 2a; $n = 10$, $t(9) = 3.663$, $p = 0.005$; Fig. 2c; $n = 11$, $t(10) = 4.578$, $p = 0.001$). Among animals that received vehicle prior to extinction training, this fear-potentiated startle response was abolished upon subsequent testing (Fig. 2a; $z = 0.255$, $p = 0.846$). Accordingly, paired t-test analysis revealed that the average percent change in startle responding was significantly decreased after extinction in vehicle-treated animals (Fig. 2b; $t(9) = 2.523$, $p = 0.033$). However, pre-extinction training treatment with BRL37344 blocked the expression of extinction learning 24 hrs later, as the average startle response to the CS-paired noise burst remained potentiated relative to noise alone presentations (Fig. 2c; $t(10) = 3.745$, $p = 0.004$). As such, the percent change pre- and post-extinction training was not significantly altered (Fig. 2d; $t(10) = 0.819$, $p = 0.432$).

LFS of the external capsule induced a significant LTP of fEPSP amplitude in slices from naïve animals (Fig. 3a; $n = 8$ slices/7 rats, $17.45\% \pm 3.73$), but not in slices from fear-conditioned (FC) animals (Fig. 3a; $n = 10$ slices/7 rats, $0.01\% \pm 3.989$). Notably, in the presence of SR58611A (5 μM), which pharmacologically enhanced LPC signaling, the LFS protocol actually resulted in LTD of fEPSPs (Fig. 3a; $n = 8$ slices/7 rats, $14.210\% \pm 3.36$). A two-way repeated measures ANOVA comparing the post-LFS amplitude in each condition

(naïve, FC, and SR58611A) across time revealed a significant interaction between these factors (Fig. 3a; $F(88, 1012) = 1.384$, $p = 0.014$). Similarly, a one-way ANOVA comparing the average percent change in amplitude following LFS was significant (Fig. 3b; $F(2, 23) = 16.269$, $p < 0.001$), and post-hoc analysis confirmed that each treatment condition differed significantly from the others (Fig. 3b; Naïve vs SR58611A: $t = 5.693$, $p < 0.001$; Naïve vs FC: $t = 3.306$, $p = 0.006$; FC vs SR58611A: $t = 2.694$, $p = 0.013$).

LFS of the external capsule in slices from behaviorally naïve rats resulted in significant LTD of LPC eIPSCs (iLTD) that lasted for at least 30 minutes (Fig. 3c; $n = 11$ slices/10 rats, $32.82\% \pm 6.93$). Notably, this iLTD was not observed in slices from FC animals (Fig. 3c; $n = 10$ slices/10 rats, $4.32\% \pm 8.28$). A two-way repeated measures ANOVA comparing eIPSC amplitude post-LFS in naïve and FC animals across time revealed a significant interaction (Fig. 3c; $F(29, 551) = 2.793$, $p < 0.001$). Similarly, a t-test comparing the average percent change in eIPSC amplitude post-LFS revealed a significant difference between the groups (Fig. 3d; $t(19) = 2.656$, $p = 0.016$).

4. Discussion

The major findings of this study are as follows: (i) selective pharmacological enhancement of LPC GABAergic synapses blocks the acquisition of fear and extinction learning; (ii) LFS of the external capsule border, which engages LPCs, produces LTP of BLA fEPSPs, and this plasticity is reversed by increasing inhibition at LPC synapses via concurrent application of a β_3 -AR agonist and occluded by fear conditioning; (iii) the same LFS protocol results in iLTD of LPC synapses, and this plasticity is also occluded by fear conditioning.

Although the importance of BLA inhibitory signaling in fear conditioning and extinction has been convincingly argued (Ehrlich et al., 2009; Lee et al., 2013), the specific contribution of the feed-forward GABAergic inputs lining the external capsule border (LPCs) to the expression of these behaviors had yet to be directly examined. Our lab recently demonstrated that β_3 -AR activation selectively potentiates LPC GABAergic inhibition in the BLA via a postsynaptic mechanism, without affecting local inhibitory synapses or glutamatergic transmission (Silberman et al., 2010). We have further shown that potentiating LPC synaptic transmission via intra-BLA microinjection of a β_3 -AR agonist significantly decreases unconditioned anxiety-like behaviors and appetitive ethanol drinking, indicating a role for this input in modulating amygdala-dependent behaviors (Silberman et al., 2010; Butler et al., 2014). Here, we report that increasing inhibitory synaptic transmission via intra-BLA microinjection of β_3 -AR agonists prior to fear conditioning blocks the expression of fear learning 24 hours later. Similarly, we report that β_3 -AR mediated potentiation of LPC GABAergic inhibition immediately before extinction training blocks subsequent expression of extinction learning. These behavioral disruptions are consistent with previous evidence that inhibiting BLA excitability decreases both fear and extinction memory acquisition (Herry et al., 2008; Hart et al., 2009; Sierra-Mercado et al., 2011).

Having demonstrated a potential role for LPC inhibition in modulating fear learning and extinction, we next sought to determine whether fear conditioning disrupts synaptic plasticity at these synapses *ex vivo*. LFS of the external capsule is known to produce LTP at

excitatory synapses (Li et al., 1998), and this LTP is occluded following fear conditioning (Tsvetkov et al., 2002; Schroeder and Shinnick-Gallagher, 2005). Presumably, this occlusion occurs because the learning-induced enhancement of this circuit shares one or more steps with electrically-induced LTP. We report that, consistent with earlier findings (Tsvetkov et al., 2002), LFS of the external capsule resulted in LTP of BLA fEPSPs in slices obtained from naïve animals, but had no impact on fEPSP amplitude following fear conditioning. Interestingly, and in corroboration with our behavioral findings, pretreatment with a β 3-AR agonist (SR58611A) also prevented electrically-induced LTP of fEPSPs, with the drug-paired LFS protocol resulting in a modest but significant LTD of BLA fEPSP amplitude. As pharmacological potentiation of synaptic transmission at LPC synapses using a β 3-AR agonist appears to reverse the polarity of LFS-induced plasticity, and *in vivo* activation of these receptors disrupts fear and extinction learning, we hypothesize that LPC synaptic transmission is decreased during fear conditioning, and that this disinhibition encourages fear learning and related long-term synaptic potentiation. In support of this hypothesis, extant research suggests that reduction of LPC signaling promotes LTP of cortical inputs onto BLA pyramidal cells and contributes to fear memory formation. For example, severing the external capsule promotes the induction of LTP from cortical areas conveying auditory and multisensory information to the BLA; however, this plasticity does not occur if feed-forward LPC inhibitory inputs onto BLA pyramidal neurons remain intact (Morozov et al., 2011). Similarly, LPC inhibition is reduced following stress exposure, resulting in increased LTP and fear conditioning (Manzanares et al., 2005). As such, we hypothesized that this same stimulation protocol would produce iLTD of LPC GABAergic synapses, and that this plasticity would be occluded by fear conditioning. Consistent with our hypothesis, we observed iLTD of LPC eIPSCs following LFS of the external capsule in BLA slices obtained from naïve animals; however, this stimulation protocol did not significantly alter signaling at LPC synapses in slices acquired from fear conditioned animals. Taken together, our findings suggest that the mechanisms required for these synapses to undergo iLTD are engaged by fear conditioning, thus occluding further electrically-induced plasticity.

Although there is ample evidence that plasticity at local inhibitory synapses contributes to fear learning (Shumyatsky et al., 2002; Bauer and LeDoux, 2004; Szinyei et al., 2007; Polepalli et al., 2010; Lange et al., 2012), these data provide the first direct evidence that fear conditioning may also involve plastic changes at LPC synapses. Interestingly, BLA medial paracapsular cells (MPCs), which line the border between the BLA and central amygdala, play a well-characterized role in extinction memory formation (Likhtik et al., 2008; Amano et al., 2011; Busti et al., 2011). As such, it seems reasonable that LPCs might likewise contribute to extinction learning, although to our knowledge this hypothesis has yet to be tested. Thus, the experiments described herein are the first to investigate whether pharmacological potentiation of LPC inhibitory signaling is sufficient to disrupt fear extinction. Our results are consistent with studies showing that inactivation of the BLA occludes cue-dependent fear extinction (Herry et al., 2008; Sierra-Mercado et al., 2011), and suggest a novel role for LPCs in extinction memory formation.

The mechanisms underlying the observed iLTD of LPC synapses are as yet unknown. Plasticity of inhibitory signaling at local BLA synapses can be mediated by an

endocannabinoid-dependent decrease in presynaptic GABA release (Azad et al., 2004). However, cannabinoid receptors are not thought to be expressed on LPCs (Katona et al., 2001), making this an unlikely mechanism of the plasticity observed herein. Another form of inhibitory synaptic plasticity has recently been characterized at local GABAergic synapses, but this mechanism requires activation of AMPA and NMDA receptors and subsequent enhancement of GABAergic transmission via retrograde signaling mechanisms (Lange et al., 2012). As we pharmacologically blocked ionotropic glutamate receptors during iLTD induction, this mechanism cannot explain our results. Additionally, we did not see any change in paired-pulse facilitation following LFS of the external capsule (data not shown); therefore, it seems likely that the observed plasticity of LPC synapses does not involve a decrease in GABA release probability. Previous research has shown that GABA_A receptors are downregulated in the BLA following fear learning (Lin et al., 2010; Lin et al., 2011), which could explain the LTD of LPC inhibition that we observed. Future studies will directly test this hypothesis.

Disrupted noradrenergic facilitation of inhibitory signaling may be one mechanism driving fear learning and related synaptic potentiation in the BLA. For instance, BLA noradrenergic signaling enhances fear memory formation (Ferry et al., 1997; LaLumiere et al., 2003; Huff et al., 2005; Tully et al., 2007), and noradrenaline release during fear learning has been shown to promote the induction of LTP by suppressing GABAergic neurotransmission at local inhibitory synapses (Tully et al., 2007). Additionally, pharmacological blockade of $\alpha 1$ -ARs, which potentiate GABA release from local inhibitory interneurons (Braga et al., 2004), has been shown to promote fear conditioning and the induction of excitatory synaptic plasticity in this region (Lazzaro et al., 2010). In this study, we have demonstrated that pharmacologically enhancing LPC inhibitory currents using a $\beta 3$ -AR agonist blocks the acquisition of fear learning *in vivo*, and the induction of BLA excitatory synaptic plasticity *ex vivo*. Thus, one possibility is that the inhibitory effect of norepinephrine at $\beta 3$ -ARs is downregulated during fear memory formation. Currently studies are underway investigating whether noradrenergic facilitation of inhibitory synaptic transmission at local and LPC synapses is disrupted by fear learning.

Taken together, our results suggest that LPCs may contribute to the regulation of BLA synaptic plasticity during the acquisition and extinction of fear learning. We have previously shown that $\beta 3$ -AR activation selectively enhances LPC-mediated GABAergic inhibition of BLA pyramidal cells, and here we extend these findings by demonstrating that pharmacological enhancement of this inhibitory circuit is sufficient to block fear and extinction learning and associated synaptic plasticity. These studies are the first to directly examine the role of LPC inhibition in fear and extinction learning. Likewise, they are the first to report the occurrence of inhibitory plasticity at LPC synapses in the BLA, and furthermore demonstrate that this plasticity is occluded by fear conditioning. As we have shown here that activation of $\beta 3$ -ARs blocks electrically-induced LTP of cortical inputs to the BLA, this input is likely disinhibited during fear conditioning, although the exact mechanism of this disinhibition is not yet known. Regardless, these data provide new evidence that BLA LPCs are involved in fear and extinction learning, and may represent a

novel synaptic substrate that contributes to the maladaptive behavioral sequelae afflicting individuals with PTSD and related psychiatric conditions.

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Highlights

- Selective pharmacological enhancement of basolateral amygdala lateral paracapsular (LPC) GABAergic synapses with a β 3-adrenoreceptor agonist blocks the acquisition of fear and extinction learning *in vivo*.
- Low-frequency *ex vivo* electrical stimulation of the external capsule border, which engages LPCs, produces long-term potentiation of BLA field EPSPs, and this plasticity is reversed by concurrent application of a β 3-adrenoreceptor agonist and occluded by fear conditioning.
- The same stimulation protocol results in long-term depression (LTD) of LPC synapses, and this plasticity is also occluded by fear conditioning.

Figure 1.

Intra-BLA microinjection the β -3-AR agonist BRL37344 (1 μ g/0.5 μ L/side) prior to fear conditioning blocked fear-potentiated startle 24 hrs later, as evidenced by no change in startle amplitude in response to the CS-paired noise (a; $p > 0.05$). In contrast, animals who received vehicle microinjections before fear conditioning exhibited a CS-potentiated startle response (a; $*p < 0.05$). Accordingly, the percent change in startle amplitude was greater among vehicle-treated animals than BRL37344-treated animals (b; $*p < 0.05$). Similarly, the β -3-AR agonist SR58611A (1 μ g/0.5 μ L/side) blocked the expression of fear-potentiated startle (c; $p > 0.05$), which remained unaffected in vehicle-treated controls (c; $*p < 0.05$). The percent change in startle response was likewise significantly increased in vehicle-treated animals relative to SR58611A-treated animals (d; $*p < 0.05$). Black circles represent microinjector tip locations (e).

Figure 2.

Intra-BLA microinjection of the β_3 -AR agonist BRL37344 (1 μ g/0.5 μ L/side) before extinction training blocked the expression of extinction learning 24 hrs later. Animals in the vehicle group acquired fear conditioning normally (a; $**p < 0.01$), and this fear response was abolished following extinction training (a; $p > 0.05$). Accordingly, the percent change in startle response was significantly decreased following extinction (b; $*p < 0.05$). In contrast, intra-BLA microinjection of BRL37344 before to extinction training blocked the expression of extinction learning 24 hrs later (c; $**p < 0.01$). As such, the percent change in fear-potentiated startle was not significantly altered pre- vs post-extinction (d; $p > 0.05$). Black circles represent microinjector tip locations (e).

Figure 3.

Low-frequency stimulation (LFS; 1 Hz, 15 min) of the external capsule lead to a significant increase in fEPSP amplitude in slices taken from naïve animals (a; $17.45\% \pm 3.73$), and this plasticity was occluded by fear conditioning (a; $0.01\% \pm 3.989$). LFS significantly decreased fEPSP amplitude when delivered after pretreatment with the β_3 -AR agonist SR58611A (5 μ M) (a; $14.210\% \pm 3.36$). Two-way repeated measures ANOVA revealed a significant interaction of time and group (a; $*p < 0.05$). Accordingly, one-way ANOVA revealed that the average percent change in fEPSP amplitude post-LFS was significantly different in each

group (b; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Similarly, LFS of the external capsule resulted in significant depression of post-LFS IPSCs (c; $32.82\% \pm 6.93$). However, this LTD was not observed in animals that had undergone fear conditioning (c; $4.32\% \pm 8.28$). Two-way repeated measures ANOVA revealed a significant interaction of time and group (c; *** $p < 0.001$), and a paired t-test confirmed that the average percent change in eIPSC amplitude post-LFS was significantly reduced following fear-conditioning (d; * $p < 0.05$).

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