Alterations of excitatory transmission in the lateral amygdala during expression and extinction of fear memory

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Abstract
Understanding the neurophysiology of fear extinction has important implications for the treatment of post-traumatic stress disorders. Here we report that fear conditioning resulted in an increase in AMPA/NMDA ratio as well as depression of paired-pulse facilitation (PPF) in neurons of the lateral nucleus of amygdala. These conditioning-induced changes in synaptic transmission were not affected by extinction training. D-cycloserine (DCS), a partial agonist at the glycine-binding site of the NMDA receptor, facilitated extinction and reversed the increase in AMPA/NMDA ratio without altering the depression of PPF when administered before extinction training. Extinction training, however, significantly increased the frequency and amplitude of miniature inhibitory post-synaptic currents and these effects were unaffected by the DCS treatment. Disruption of AMPA receptor endocytosis with a synthetic peptide containing a short C-terminal sequence of GluR2 (YKEGYNVYG, GluR2C) specifically blocked DCS-induced reversal of AMPA/NMDA ratio and the facilitation of extinction. These results suggest that extinction training mainly increases inhibitory transmission leaving conditioning-induced excitatory association unaltered. DCS does not affect inhibitory transmission but reverses the conditioning-induced post-synaptic memory trace when administered before extinction training.

Key words: Amygdala, extinction, fear conditioning, post-traumatic stress disorder.

Introduction
In the lateral amygdala (LA) and basolateral amygdala (BLA), excitatory pyramidal neurons use glutamate as a neurotransmitter (McDonald & Augustine, 1993; Sah et al. 2003). During fear conditioning, LA neurons receive conditioned stimuli (CS) from the auditory thalamus and cortex and aversive unconditioned stimuli (US) from the thalamus. Associative activation of CS and US pathways which accompany behavioural learned fear results in long-term potentiation (LTP) of synaptic transmission from auditory thalamus and cortex to LA (McKernan & Shinnick-Gallagher, 1997; Rogan et al. 1997), occluded LTP-induced presynaptic enhancement (Tsvetkov et al. 2002) and increased synaptic GluR1 subunit of AMPA receptors (AMPARs) (Rumpel et al. 2005; Yeh et al. 2006). Interference with synaptic incorporation of GluR1 receptors in LA impairs LTP and fear memory formation (Rumpel et al. 2005; Sigurdsson et al. 2007). Extinction is a behavioural paradigm in which the conditioned response gradually disappears if animals receive the cue only without pairing with a shock (Myers & Davis, 2002). The majority of patients with post-traumatic stress disorders (PTSD) exhibit long-lasting re-experience of traumatic events and, it is thought that dysfunction of fear extinction plays an important role in the development of clinical symptoms of PTSD. Recently, it has been shown that a partial agonist at the glycine-binding site of the NMDA receptor (NMDAR) D-cycloserine (DCS) facilitated extinction of conditioned fear and cocaine-induced place preference in rats (Botreau et al. 2006; Ledgerwood et al. 2003, 2004; Walker et al. 2002) and enhanced exposure therapy of acrophobia (Davis et al. 

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2006; Ressler et al. 2004), social anxiety (Guastella et al. 2008) and obsessive–compulsive disorders (Kushner et al. 2007) in clinical trials.

Currently, there are two hypotheses concerning the mechanism of how DCS facilitates extinction. First, activation of a glycine site by DCS enhanced the function of NMDARs. Therefore, the facilitation of extinction by DCS could result from its enhancement of NMDA responses and subsequently the consolidation of extinction memory. Alternatively, DCS could facilitate extinction by unlearning because conditioning–induced increase in GluR1 was found to be reversed when DCS was infused into the amygdala before extinction training (Mao et al. 2006). Consistent with the latter hypothesis, rats failed to show reinstatement when DCS was given immediately after extinction training (Ledgerwood et al. 2004). However, failure to display reinstatement after DCS treatment does not necessarily mean the effect results from unlearning. It could be due to enhanced inhibitory conditioning of the context during extinction training. Further experiments by Woods & Bouton (2006) revealed that DCS did not eliminate renewal of conditioned fear suggesting that the original association was not impaired. Thus, it remains to be determined whether DCS facilitates extinction by erasing memory trace and/or by enhancing inhibitory learning. Despite these hypotheses, the effect of DCS on extinction has not been assessed electrophysiologically before. In the present study, we aim to unravel the effects of DCS on the excitatory and inhibitory synaptic transmission in the LA neurons and explore their relationship with the extinction of conditioned fear.

Methods

Animals

All procedures were approved by the Institutional Animal Care and Use Committee of the College of Medicine, National Cheng-Kung University, Taiwan. Animals were housed in cages of four rats per cage in a temperature (24°C)-controlled animal colony, pelleted rat chow and water were available ad libitum. The rats were maintained on a 12-h light–dark cycle (lights on 07:00 hours). All behavioural procedures took place during the light cycle.

Surgery

Male Sprague–Dawley rats (175–200 g), were anaesthetized with sodium pentobarbital (50 mg/kg i.p.), and mounted on a stereotaxic apparatus and a cannula of 22-gauge stainless-steel tubing was implanted into the LA or BLA [anteroposterior (AP), –2.8 mm; mediolateral (ML), ±4.5 mm; dorsoventral (DV), –7.0 mm]. A 28-gauge dummy cannula was inserted into each cannula to prevent clogging. A TAT-conjugated peptide (GluR2ΔY, YKEGYNVVG) designed to impair AMPAR endocytosis was dissolved in 0.9% NaCl and infused into the LA or BLA (15 pmol/side) bilaterally 30 min before extinction training. The control peptide had the sequence AKEGANVAG (GluR2ΔA). The dose was chosen with regard to Brebner et al. (2005).

Behavioural apparatus and procedures

Expt 1: fear conditioning

Rats were trained and tested in a stabilimeter device. Behavioural experiments of fear conditioning were performed in standard operant chamber (San Diego Instruments, USA). The acoustic startle stimulus was a 50 ms white-noise at 95 dB intensity. The visual CS was a 3.7 s light produced by an 8 W fluorescent bulb attached to the back of stabilimeter. The US was a 0.6 mA footshock of 0.5 s duration. Rats were placed in the startle test boxes for 10 min and returned to their home cages on three consecutive days to habituate them to the test chamber and to minimize the effect of contextual conditioning. Rats were placed in the startle boxes and received 10 light-footshock pairings with an inter-trial interval (ITI) of 2 min. Unpaired rats received the same number of CS and US presentations, but in an unpaired pseudo-random fashion. The rats were tested for fear-potentiated startle 24 h after conditioning. The test involved 30 startle-eliciting noise bursts presented alone (noise-alone trial) and 30 noise bursts presented 3.2 s after onset of the 3.7 s light (light-noise trials). The two trial types were presented in a balanced mixed order (ITI, 30 s). One hour after the test, the rats were sacrificed and amygdala slices were prepared for electrophysiological recordings and biochemical measurements.

Expt 2: fear extinction

Rats were trained with 10 light-shock pairings and retention of memory was tested 24 h later (test 1). On day 3, the rats received three sessions of 10 light-alone presentations (extinction training) and retention of memory was tested 24 h after extinction training (test 2). Context exposure control rats were exposed to the startle chamber for the same amount of time without receiving light-alone trials. DCS (20 mg/kg) or vehicle was injected intraperitoneally (i.p.) into the rats 30 min before extinction training. One hour after the test 2, the rats were sacrificed and amygdala slices...
were prepared for electrophysiological recordings and biochemical measurements.

Slice preparation

Male Sprague–Dawley rats aged 5–8 wk were decapitated and their brains rapidly removed and placed in cold oxygenated, artificial cerebrospinal fluid (aCSF) solution. Subsequently, the brain was hemisected and cut transversely posterior to the first branch and anteriorly to the last branch of the superior cerebral vein. The resulting section was glued to the chuck of a Vibroslice tissue slicer. Transverse slices of 500 μm thickness were cut and the appropriate slices placed in a beaker of oxygenated aCSF at room temperature for at least 1 h before recording. The aCSF solution had the following composition (in mM): NaCl 117, KCl 4.7, CsOH; the final osmolarity was adjusted to 290–300 mOsm by adding sucrose. Records were low-pass-filtered at 2.5–20 kHz and digitized at 5–50 kHz. The aCSF was bubbled continuously with 95%O2/5%CO2 (pH 7.4).

Whole-cell patch-clamping recordings

Whole-cell patch-clamp recordings were made from the LA neurons. Excitatory post-synaptic currents (EPSCs) were evoked at 0.03 Hz by extracellular stimulation of fibres emerging from the internal capsule which originate in the medial geniculate nucleus of the thalamus and project monosynaptically to the LA using a bipolar electrode. Patch electrodes were pulled from thick-wall glass capillary (0.86 mm i.d., 1.5 mm o.d.) to a tip resistance of 3–5 MΩ. The composition of the internal solution was (in mM): K-glucuronate 140, KCl 10, EGTA 1, phosphocreatine 10, Mg-ATP 4, Na-GTP 0.3, Heps 10 for recording neuronal properties and action potentials. The composition of the internal solution was (in mM): caesium methane-sulfonate 128, NaCl 20, EGTA 1, CaCl2 0.3, MgCl2 1, Na-ATP 3, Na-GTP 0.4, Heps 10 for recording AMPA/NMDA ratio. The final pH of the internal solution was adjusted to 7.3 by adding 1 M KOH or CsOH; the final osmolarity was adjusted to 290–300 mOsm by adding sucrose. Records were low-pass-filtered at 2.5–20 kHz and digitized at 5–50 kHz. The signal was monitored and recorded with an Axopatch 200B amplifier. Online analysis and control of experimental acquisition was accomplished via a 586 (Intel)-based PC clone and a Digidata 1320 computer interface. AMPAR-mediated EPSC (AMPA EPSC) was evoked when the neurons were voltage-clamped at −70 mV, whereas NMDAR-mediated EPSC (NMDA EPSC) was determined as current amplitude at 50 ms after peak EPSC amplitude at a holding potential of +40 mV (Du et al. 2008). AMPA/NMDA ratio was also measured by recording EPSCs at +40 mV before and after application of the NMDAR blocker D-APV (50 μM). NMDA EPSC was calculated by subtracting the response in the presence of D-APV from control response without adding D-APV. The peak of the AMPA EPSC was divided by the peak of the NMDA EPSC to yield an AMPA/NMDA ratio (Ungless et al. 2001).

Data analysis

A single-factor ANOVA and post-hoc comparisons were used to analyse the differences in fear-potentiated startle and EPSCs among naive, unpaired and paired groups. Unpaired t test was used to analyse differences of AMPA/NMDA ratio and paired-pulse facilitation (PPF) between vehicle- and DCS-treated rats. All values are mean ± S.E.M. p values <0.05 were considered to be statistically significant.

Results

Alterations of excitatory transmission in the amygdala after fear conditioning

Rats were randomly assigned to naive, paired and unpaired groups. Rats in the paired group received 10 light-shock pairings and learned that light appearance predicted subsequent shock presentation. The unpaired group received 10 lights and 10 shocks in a pseudo-random manner and consequently did not learn light-shock association. Naive rats did not receive any training. As a result of fear learning, fear-potentiated startles were significantly elevated in the paired rats compared to those of unpaired and naive controls (Fig. 1a). An ANOVA comparing the difference between light-noise and noise-alone showed a significant effect of groups (F2,41 =101.3, p <0.001). Newman–Keuls post-hoc analysis revealed that paired group was significantly different from both unpaired and naive groups (p<0.001) (Fig. 1a). Mean baseline startle was not different among groups (p>0.1).

Whole-cell recordings were made from the soma of visually identified pyramidal-like neurons located in the LA. Neurons were identified as projection neurons based on their morphology in caesium methane-sulfonate-containing electrodes. In potassium glucose-containing electrodes, these neurons were also identified by their intrinsic electrophysiological properties, in particular spike frequency adaptation in response to depolarizing current injection. We determined whether the excitatory synaptic transmission in the LA neurons was altered after fear conditioning by measuring the relative contribution of AMPAR and
NMDAR to the EPSCs which could minimize the effect of slice-to-slice variability and has been proven as a sensitive assay for detecting difference in the glutamatergic synaptic strength (Bellone & Luscher, 2006; Clem & Barth, 2006; Kourrich et al., 2007; Ungless et al. 2001). AMPA EPSC was evoked when the neurons were voltage-clamped at −70 mV whereas NMDA EPSC was determined as current amplitude at 50 ms after peak EPSC amplitude at a holding potential of +40 mV (Du et al. 2008). In naive and unpaired rats, the AMPA/NMDA ratios were 1.43 ± 0.11 (n = 6) and 1.34 ± 0.14 (n = 6), respectively. The ratio was significantly higher in the paired rats (3.09 ± 0.2, n = 6, p < 0.01) (Fig. 1b).

We also determined AMPA/NMDA ratio by measuring EPSCs at +40 mV before and after application of the NMDAR blocker D-APV (50 µM). NMDA EPSC was calculated by subtracting the response in the presence of D-APV from control response without adding D-APV. The peak of the AMPA EPSC was divided by the peak of the NMDA EPSC to yield the AMPA/NMDA ratio (Ungless et al., 2001). Consistent with the above result, the paired rats exhibited a significantly higher AMPA/NMDA ratio (1.04 ± 0.11, n = 5) than the unpaired rats (0.55 ± 0.08, n = 5, p < 0.01).

PPF is a measure of short-term plasticity widely used to probe for changes in presynaptic function because changes in PPF are inversely related to transmitter release. To determine whether the increased synaptic strength recorded 1 d after fear conditioning involved a presynaptic mechanism, we analysed PPF in slices from naive, unpaired and paired rats. Ratios of the amplitude of the second EPSC to the amplitude of the first EPSC were examined at different interpulse intervals. PPF at 30- and 60-ms intervals in the paired group was less significant (30 ms: 0.81 ± 0.10; 60 ms: 0.96 ± 0.10, p < 0.05) than those of naive (30 ms: 1.44 ± 0.16; 60 ms: 1.29 ± 0.08) and unpaired (30 ms: 1.27 ± 0.07; 60 ms: 1.33 ± 0.10) rats (Fig. 1c). This result suggests that enhanced synaptic efficacy after fear conditioning is mediated at least in part by an increase in presynaptic release probability.

We next determined whether fear conditioning affected NMDA EPSC which was isolated in the presence of AMPA (CNQX 10 µM) and GABA_A receptor (bicuculline 10 µM) antagonists. In agreement with a previous observation (Zinebi et al. 2003), fear conditioning reduced the amplitude of NMDA EPSC (Fig. 2b) whereas the voltage-dependence of NMDA EPSC was not altered (Fig. 2d).

Alterations of excitatory transmission in the amygdala after fear extinction

We determined the changes of EPSC following the extinction of fear memory. Rats were trained with
10 light-shock pairings and retention of memory was tested 24 h later (test 1). On day 3, the rats received three sessions of 10 light-alone presentations (extinction training) and retention of memory was tested 24 h after extinction training (test 2). DCS (20 mg/kg) or vehicle was injected i.p. into the rats 30 min before extinction training. Figure 3 shows that vehicle- and DCS-treated rats exhibited significant reduction of fear-potentiated startle after extinction training. However, fear-potentiated startle in the rats treated with DCS in test 2 was less than that of the vehicle controls (\( p < 0.05 \)), confirming facilitation of fear extinction by DCS (Ledgerwood et al. 2003; Mao et al. 2006; Walker et al. 2002).

Amygdala slices from vehicle- and DCS-treated extinction rats were prepared 1 h after test 2.

**Fig. 2.** Fear conditioning reduces the amplitude of NMDA EPSC without altering its voltage-dependency. (a) Current-voltage plot of the NMDA EPSC. NMDA EPSCs were recorded in the presence of CNQX (10 \( \mu M \)) and bicuculline (10 \( \mu M \)) at holding potentials of \(-80 \) mV to \(+50 \) mV in unpaired, paired and extinction rats. (b) Values of the amplitude of NMDA EPSC in unpaired, paired and extinction rats (* \( p < 0.05 \) vs. unpaired rats).

**Fig. 3.** Reversal of conditioning-induced increase in AMPA/NMDA ratio by extinction training in combination with DCS treatment. (a) Rats received 10 light-shock pairings and retention of memory was assessed 24 h later (test 1). On day 3, the rats were injected intraperitoneally with DCS (20 mg/kg, \( n = 6 \)) or vehicle (\( n = 6 \)) 30 min before extinction training (three blocks of 10 presentations of light-alone trials). Retention of memory was assessed 24 h after extinction training (test 2) (*** \( p < 0.001 \) vs. test 1). (b) Plot of AMPA/NMDA ratio in the vehicle- and DCS-treated extinction rats (** \( p < 0.01 \) vs. vehicle; scale 50 ms, 100 pA). (c) Plot of paired-pulse facilitation in the vehicle- and DCS-treated extinction rats (scale 50 ms, 40 pA).
A no-injection group which received conditioning followed by extinction training but without drug injection served as control. As illustrated in Fig. 3b, the AMPA/NMDA ratio in DCS-treated extinction rats (1.48 ± 0.07, n = 7) was significantly lower than that of vehicle-treated rats (3.04 ± 0.26, n = 7, p < 0.01) and no-injection extinction rats (2.92 ± 0.18, n = 7, p < 0.01). AMPA/NMDA ratios were not significantly different among paired (3.16 ± 0.21, n = 7), no-injection and vehicle-treated extinction rats (p > 0.2), suggesting that extinction did not affect conditioning-induced increase in AMPA/NMDA ratio. In addition, there was no difference in AMPA/NMDA ratio among naive, unpaired and DCS-treated extinction rats (F(2,12) = 0.16, p > 0.5), indicating the reversal of conditioning-induced increase in the AMPA/NMDA ratio by the combination of DCS with extinction training.

Analysis of PPF at 30-, 60- and 150-ms intervals revealed no difference in slices from the paired, DCS- and vehicle-treated extinction rats (p > 0.05) (Fig. 3c). The result suggests that neither extinction itself nor extinction in combination with DCS altered conditioning-induced depression of PPF.

In contrast to AMPA/NMDA ratio, conditioning-induced reduction in NMDA EPSC could be reversed after extinction training (Fig. 2b).

**Effects of disruption of AMPAR endocytosis on extinction**

A synthetic peptide containing a short C-terminal sequence of GluR2 (869-877, GluR2<sup>3Y</sup>) has been shown to block LTD, depotentiation and AMPAR endocytosis in the hippocampus, nucleus accumbens and amygdala (Ahmadian et al. 2004; Brebner et al. 2005; Dalton et al. 2008; Kim et al. 2007).

When GluR2<sup>3Y</sup> was fused to the cell membrane transduction domain of the HIV-1 Tat protein (Tat- GluR2<sup>3Y</sup>), it became membrane permeable and was able to impair extinction of fear memory. To determine whether GluR2<sup>3Y</sup> actually has an effect on the LA neurons, we tested its effect on NMDA-induced internalization of GluR2.Amygdala slices were prepared and incubated with aCSF, GluR2<sup>3Y</sup> (10 μM) or GluR2<sup>3A</sup> (869-877, GluR2<sup>3A</sup>) (10 μM), a control peptide which lacks an effect on AMPAR endocytosis for 60 min.

Figure 4 shows that the levels of internalized GluR2 were not significantly different among these three groups. NMDA application (20 μM, 3 min) caused an increase in internalized GluR2 (164.5 ± 11.9% of aCSF control, n = 4). Co-administration of NMDA with GluR2<sup>3Y</sup> blocked the effect of NMDA (107.5 ± 11.2%, n = 4, p < 0.01). In contrast, GluR2<sup>3A</sup> (10 μM) had no effect on NMDA-induced GluR2 internalization (101.0 ± 6.6% of aCSF control, n = 4). This result demonstrates that GluR2<sup>3Y</sup> is able to block NMDA-induced GluR2 internalization in the *in-vitro* amygdala slices.

We performed behavioural assessment to determine whether Tat-GluR2<sup>3Y</sup> influenced extinction. Rats were divided into four groups: rats that received Tat-GluR2<sup>3Y</sup> (15 pmol in 0.8 μl saline per side) or Tat-GluR2<sup>3A</sup> followed by DCS (20 mg/kg i.p.) or vehicle 30 min before extinction training. Memory retention was assessed 24 h after extinction training. A two-way ANOVA revealed no effect of group (Tat-GluR2<sup>3Y</sup> vs. Tat-GluR2<sup>3A</sup>, p = 0.17) and drug treatment (DCS vs. vehicle, p = 0.24) but a significant effect of interaction (p < 0.05). Fear-potentiated startle in the Tat-GluR2<sup>3A</sup>-DCS rats was significantly less than that in the Tat-GluR2<sup>3Y</sup>-DCS rats (p < 0.05) (Fig. 5a). Furthermore, startle potentiation in the Tat-GluR2<sup>3Y</sup>-vehicle rats was comparable with that of Tat-GluR2<sup>3A</sup>-vehicle rats (p = 0.47) indicating that Tat-GluR2<sup>3Y</sup> was without effect in the absence of DCS. Interestingly, in the
Tat-GluR2<sub>3Y</sub> rats, startle potentiation in the DCS-treated rats was comparable with that of vehicle-treated rats \( (p = 0.31) \) indicating that Tat-GluR2<sub>3Y</sub> specifically blocked the effect of DCS on extinction.

We determined whether Tat-GluR2<sub>3Y</sub> affected excitatory transmission by recording the AMPA/NMDA ratio. In order to keep the integrity of the slices, Tat-GluR2<sub>3Y</sub> was administered i.p. Thus, rats received i.p. injection of saline or DCS (20 mg/kg) followed 30 min later by extinction training. Tat-GluR2<sub>3Y</sub> (3 μmol/kg i.p.) or Tat-GluR2<sub>3A</sub> (3 μmol/kg i.p.) was injected 60 min before the administration of DCS. Memory retention was assessed 24 h after extinction training. A two-way ANOVA revealed no effect of group (Tat-GluR2<sub>3Y</sub> vs. Tat-GluR2<sub>3A</sub> \( p = 0.09 \)) and drug treatment (DCS vs. vehicle, \( p = 0.14 \)) but a significant effect of interaction \( (p < 0.05) \). Startle potentiation in the Tat-GluR2<sub>3A</sub>-DCS rats was significantly less than that in the Tat-GluR2<sub>3Y</sub>-DCS rats \( (p < 0.05) \) (Fig. 6a).

Amygdala slices from each group were prepared 24 h after extinction training. Figure 6b shows that the AMPA/NMDA ratio was significantly lower in DCS + Tat-GluR2<sub>3A</sub>-treated rats compared to the DCS + Tat-GluR2<sub>3Y</sub> groups \( (p < 0.001) \). These results suggest that Tat-GluR2<sub>3Y</sub> itself has no effect but specifically blocks the effect of DCS on the AMPA/NMDA ratio.

**Effect of DCS on inhibitory transmission**

Rats were trained as described above and received an i.p. injection of DCS (20 mg/kg) or vehicle 30 min before extinction training. We compared miniature inhibitory post-synaptic currents (mIPSCs) among slices from the DCS-, vehicle-treated extinction, and paired (no extinction training) rats. As shown in Fig. 7, both mIPSC frequency and amplitude were lower in slices from the paired animals than those from the DCS- and vehicle-treated extinction animals \( (p < 0.001) \), suggesting that extinction training increased the inhibitory tone. It should be noted that both frequency and amplitude of the mIPSC were not different between DCS- and vehicle-treated extinction animals indicating that DCS was without effect on mIPSC.

**Discussion**

In the present study, we showed that AMPA/NMDA ratio in the thalamo-LA synapses was elevated in the conditioned but not unpaired rats. In addition, PPF of EPSC was depressed in the conditioned rats indicating that synaptic transmission underwent LTP after fear conditioning and the processes involved both pre- and post-synaptic mechanisms. These results were consistent with previous reports that neuronal changes mediating association between the cue and the aversive stimuli occur in the amygdala (Humeau et al. 2003; McKernan & Shinnick-Gallagher, 1997; Rogan et al. 1997; Sigurdsson et al. 2007).
Because of clinical importance as an animal model of exposure therapy for the treatment of anxiety disorders, over the past several years extinction mechanisms have received increasing attention. In theory, there are three major models concerning the mechanisms of extinction. Extinction could be due to (1) devaluation of the US representation (Rescorla, 1973); (2) unlearning or erasure of the CS-US association (Rescorla & Wagner, 1972), or (3) formation of a new inhibitory CS/no-US association (Bouton & King, 1983; Bouton & Peck, 1989). Although extinction training significantly reduced fear-potentiated startle, notably we showed here that it affected neither conditioning-induced increase in AMPA/NMDA ratio nor the depression of PPF. In addition, extinction training resulted in an increase in frequency and amplitude of IPSCs. These results favour the view that extinction is a result of increased inhibitory learning but not erasure of the original excitatory association.

It is known that NMDAR plays a critical role in the extinction of fear memory and in the acquisition of new fear memory in previously conditioned animals (Falls et al. 1992; Lee & Kim, 1998). Consistent with a previous report (Zinebi et al. 2003), we observed that fear conditioning reduced the amplitude of NMDA EPSC. Interestingly, conditioning-induced reduction in NMDA EPSC could be reversed after extinction training, a result in contrast to that of the AMPA/NMDA ratio. PPF, an index of presynaptic transmitter release, was unaltered after extinction training suggesting a post-synaptic change of NMDARs. It is speculated that during fear conditioning excitatory synaptic transmission increased concomitantly with a decrease in inhibitory transmission. Therefore, the reduction of NMDA EPSC could be a mechanism that protects against excitotoxicity (Zinebi et al. 2003). After extinction training, excitatory synaptic transmission remained elevated which could be counteracted by the increase in inhibitory transmission leaving the compensatory alteration of NMDARs unnecessary.

By binding to the strychnine-insensitive glycine recognition site, DCS enhances NMDAR-mediated synaptic response in the LA. Therefore, the facilitation of fear extinction by DCS could, theoretically, be due to its enhancement of inhibitory learning. In the present study, we found that conditioning-induced increase in AMPA/NMDA ratio was reversed by DCS in combination with extinction training. Analysis of frequency and amplitude of mIPSC revealed that there was no difference between DCS- and vehicle-treated extinction rats indicating that DCS did not affect GABAergic inhibitory transmission. Furthermore, Tat-GluR2ΔY, a synthetic peptide which inhibits AMPAR endocytosis, blocked only the additional reduction caused by DCS treatment, rather than returning the fear-potentiated level to that of fear-conditioned
animal without extinction. These results coupled with the observation that DCS promoted receptor internalization (Mao et al. 2006) imply that DCS facilitates extinction by erasing AMPAR expression but not by enhancing the inhibitory extinction memory. These results are in agreement with the observation that animals exhibit less reinstating effect after been treated with DCS before extinction training (Ledgerwood et al. 2004).

However, a recent report suggests a different view showing that the DCS-mediated extinguished response could be renewed when the rats were returned to the context where fear had been conditioned (Woods & Bouton, 2006). Somatosensory input is strong and it is likely that US-alone presentations cause robust depolarization to LA neurons and then to the BLA neurons. Projections from BLA to the mPFC synapse on both principal pyramidal neurons and parvalbumin-immunoreactive GABAergic interneurons (Gabbott et al. 2006). The inhibitory interneurons innervate the soma and initial axonal segments of mPFC pyramidal cells leading to the inhibition of spontaneous firing which in turn disinhibits LA neurons and reinstates the fear response (Buffalari & Grace, 2007; Rosenkranz et al. 2003). The reinstatement was attenuated by DCS because conditioning-induced increase in AMPA/NMDA ratio was eliminated. However, the increase in glutamate release, elicited by previous conditioning which was unaffected by DCS treatment, may drive downstream structures to exhibit fear response when the rats are returned to the context in which fear has been conditioned (renewal effect).

An interesting characteristic of DCS is that systemic administration of DCS facilitated extinction at the first time but not the second time (Langton & Richardson, 2008). One possible explanation for the failure of DCS to enhance re-extinction after re-training is that DCS pre-treatment induced the development of tolerance (Richardson et al. 2004). Alternatively, neural circuitry associated with re-extinction is different from initial extinction. In view of the fact that during re-extinction mPFC lesions caused greater resistance to extinction compared to mPFC lesions during initial extinction, the mPFC may play a more prominent role in re-extinction than initial extinction whereas amygdala is not required for re-extinction ( Morgan et al. 2003). In this respect, our result showing that inhibition of AMPAR endocytosis in the amygdala specifically blocks DCS-mediated facilitation of extinction seems to be consistent with the latter explanation.

In summary, using electrophysiological and behavioural assessments, we tackled fundamental issues regarding the extinction of conditioned fear. First, we
provided direct evidence that synaptic potentiation associated with fear conditioning was the result of a combination of pre- and post-synaptic modifications of excitatory and inhibitory transmission. Second, we found that conditioning-induced increase in AMPA/NMDA ratio and depression of PPF was unaffected by extinction training. Third, we found that DCS treatment plus extinction training reversed conditioning-induced increase in AMPA/NMDA ratio without affecting the depression of PPF. These results suggest that DCS does partially erase the original excitatory association. The context-dependent renewal of learned fear after DCS treatment could be explained by the inability of DCS to reverse conditioning-induced increase in presynaptic transmitter release.

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Statement of Interest

None.

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