J Pharmacol Sci **123**, 267 – 278 (2013)

Journal of Pharmacological Sciences

Full Paper

Synaptic Modulation via Basolateral Amygdala on the Rat Hippocampus–Medial Prefrontal Cortex Pathway in Fear Extinction

Sumitaka Inoue[1], Hidekazu Kamiyama[1], Machiko Matsumoto[1], Yoshiki Yanagawa[1], Sachiko Hiraide[1], Yasuhiro Saito[1], Kei-ichi Shimamura[1], and Hiroko Togashi[1,*]

[1]*Department of Pharmacology, School of Pharmaceutical Science, Health Sciences University of Hokkaido, Ishikari-Tobetsu 061-0293, Japan*

Received July 22, 2013; Accepted September 23, 2013

Abstract. The present study elucidated the functional role of modulatory effects of basolateral amygdala (BLA) on synaptic transmission in the rat hippocampus–medial prefrontal cortex (mPFC) pathway, compared with the hippocampal dentate gyrus (DG). Exposure to conditioned fear stress (CFS) or prior BLA activation enhanced tetanus-induced long-term potentiation (LTP) in DG. A similar synaptic response was found by low frequency stimulation (LFS) prior to tetanus. In mPFC, they did not affect LTP, but prior BLA activation, as well as pretreatment with the *N*-methyl-D-aspartate (NMDA)-receptor antagonist MK-801 (0.1 mg/kg, i.p.), suppressed LFS-primed LTP. This BLA-mediated synaptic pattern was mimicked by synaptic changes observed in the fear extinction process; prior BLA activation suppressed the synaptic potentiation responsible for extinction retrieval and attenuated decreases in fear-related freezing behavior. These data suggest that LFS-primed LTP in mPFC is related to the neural basis of extinction. Extinction-related synaptic potentiation did not occur in a juvenile stress model that exhibited extinction deficit. In addition, LFS-primed LTP was suppressed in this model, which was reversed by the NMDA-receptor agonist D-cycloserine (15 mg/kg, i.p.). These findings suggest that modulatory effects of BLA on synaptic function in the hippocampus–mPFC pathway play a significant role in fear extinction in rats.

Keywords: basolateral amygdala, synaptic potentiation, medial prefrontal cortex, extinction retrieval, low frequency stimulation

Introduction

Synaptic historical events can influence the subsequent efficacy of synaptic plasticity, which is known as synaptic metaplasticity (1, 2). This synaptic metaplasticity occurred not only by electrophysiological manipulations but also by exposure to stress. For example, unconditioned fear stress such as elevated platform stress or exposure to novel circumstances suppressed high frequency stimulation (tetanus)-induced long-term potentiation (LTP) in the hippocampal CA1 field (3, 4); however, they enhanced the magnitude of LTP in another hippocampal subregion, the dentate gyrus (DG) (5, 6). These differential metaplastic changes mimic those induced by basolateral amygdala (BLA) activation; electrical stimulation of BLA suppressed LTP in the CA1 field (7) but enhanced LTP in DG (7 – 9). Furthermore, lesions or pharmacological manipulation of BLA altered stress-induced synaptic metaplasticity and hippocampal-dependent memory function (7, 10). These findings indicate that BLA, through its projection to the hippocampus (11), can modulate synaptic metaplasticity and mnemonic processes in a hippocampal subregion–specific manner.

Anatomical studies revealed that BLA sends a direct excitatory projection not only to the hippocampus but also to the medial prefrontal cortex (mPFC) (12). The projection from BLA to mPFC forms a neural circuit that has been implicated in cognitive and emotional processes, including memory formation (13, 14). A network of

*Corresponding author. hirot@hoku-iryo-u.ac.jp
Published online in J-STAGE on November 2, 2013
doi: 10.1254/jphs.13123FP

these brain structures such as BLA, mPFC, and hippocampus has been identified in mediating encoding and expression of fear extinction (15, 16). Particularly, the hippocampus–mPFC (HPC-mPFC) pathway with its relationship to the BLA is considered to be a crucial element of the pathophysiology of psychiatric disorders such as depression and anxiety (17). Recently, Richter-Levin and Maroun (18) reported that BLA activation influenced synaptic plasticity, including metaplastic changes, in the HPC-mPFC pathway in anesthetized rats. However, the functional significance of the modulatory effects of BLA remains to be clarified.

Here we elucidated the significant role of BLA modulation of synaptic function in the HPC-mPFC pathway by focusing on mnemonic processes, fear retrieval, and fear extinction. We first examined the effects of BLA activation or exposure to conditioned fear stress (CFS) on LTP in mPFC, compared with those in the hippocampal DG subregion. On the basis of previous reports that synaptic metaplasticity, i.e., LTP suppression, by LFS prior to tetanus in CA1 is regulated by BLA (19, 20), we next examined the possible involvement of BLA modulation in LFS-primed LTP in mPFC. Finally, we investigated the effects of BLA activation on synaptic changes responsible for extinction retrieval of context-dependent fear memory. Considering the previous findings that a stressful event in early life causes long-lasting alteration in emotional circuits (21, 22), modulatory effects of BLA on synaptic function in mPFC was also examined in a model of juvenile stress [aversive footshock (FS)] exposure during the third postnatal week (3wFS) that exhibited extinction deficit (23, 24).

Materials and Methods

Animals

Adult male Wistar rats (11 – 14-week-old) were used. Rats were bred in our laboratory, with the exception of the first breeder adult rats supplied by Sankyo Labo Service, Ltd. (Tokyo). Weaning from the mother occurred on postnatal day (PND) 28. Rats were housed in a room with a 12-h light/dark cycle with constant temperature (21°C ± 2°C). All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Animal Research Committee of Health Sciences University of Hokkaido and were in accordance with National Institutes of Health guidelines.

Juvenile stress model

Rat pups were divided into two groups, i.e., non-footshock (Non-FS) control group and a juvenile stress model (24). Briefly, pups received FS as an early postnatal stressor during the third postnatal week (PND 21 – 25, 3wFS group) in a FS box [32 × 25 × 28 cm (height); Freeze Frame-41, Neuroscience Co., Ltd., Tokyo] combined with an automated analysis system (LimeLight-1, Neuroscience Co., Ltd). The FS box was composed of acrylic walls, an aluminum bottom, and a grid floor (diameter of rods, 0.4 cm; spacing, 1.1 cm). This FS box was tightly encased in a larger, soundproof box [78 × 80 × 80 cm (height)]. Pups were acclimated to the FS box for 5 min and subjected to five FS (shock intensity, 1.0 mA; intershock interval, 28 s; shock duration, 2 s). FS stimulation occurred once each day for 5 days. We chose at least two pups from each litter to serve as controls. Non-FS control group was allowed to remain in the FS box for same time period (12.5 min) without FS stimuli for 5 days. Rats were housed 2 – 4 pups per cage with a 12-h/12-h light/dark cycle under constant temperature after weaning (PND 28). During the post-adolescent period (11 – 14 weeks), electrophysiological experiments were performed under anesthesia or freely moving conditions.

Electrophysiological experiments

Evoked potential in mPFC by stimulation of the ventral hippocampus: A recording electrode was stereotaxically lowered into the prelimbic area of mPFC (coordinates: 3.3 mm anterior, 0.8 mm lateral from the bregma, 3.3 mm ventral from the cortical surface), and a bipolar stimulating electrode with a tip separation of 500 μm was placed in the CA1/subicular region of the ventral hippocampus (coordinates: 6.0 mm posterior, 5.6 mm lateral from the bregma; 4.5 – 6.5 mm ventral from the cortical surface) under urethane (1 g/kg, i.p.) anesthesia. The recording electrode and stimulating electrode were implanted in the right ipsilateral side. The intensity of the test stimulation was adjusted for each rat to elicit a population spike amplitude (PSA) of approximately 60% of the maximum amplitude. Electrode positions were optimized to record maximal field responses evoked at frequency (250-μs duration) following electrophysiological criteria (25). The PSA in mPFC stimulated by the CA1/subicular region (frequency, 0.1 Hz; pulse duration, 250 μs; stimulus interval, 30 s) was obtained from five stimuli and was recorded every 2.5 min with a Power Lab Data Acquisition System (AD Instruments, Pty., Ltd., Bella, Australia). LTP in mPFC was induced by high frequency (tetanic) stimulation (Tetanus: 2 sets of 10 trains; each train consisted of 50 pulses at 250 Hz, intertrain interval of 10 s, interest interval of 1 min) of the CA1/subicular region. Low frequency stimulation (LFS: 900 pulse at 1 Hz) was given for 15 min prior to LTP-inducing tetanic stimulation.

For determining the evoked potential in mPFC under

freely moving conditions, the following procedures were used: a recording electrode and a stimulating electrode was lowered into the mPFC and CA1/subicular region, respectively, under pentobarbital (50 mg/kg, i.p.) anesthesia. The electrodes were anchored by small, electrically grounded screws and affixed to the skull with quick, self-curing acrylic resin (UNIFAST; GC Corp., Tokyo). After surgery, each rat was singly housed to protect the implanted electrode. Five days later, electrophysiological experiments were performed under freely moving conditions following the experimental protocol (CFS and extinction process). Stimulating electrodes were connected through a cable to an electric stimulator (SEN-3301; Nihon Kohden, Tokyo) and an isolator (SS-202J, Nihon Kohden) using a cable. Another cable from the recording electrode was connected to an amplifier (gain 1000 ×, band-pass 0.08 – 10 KHz; MEG-2100 or S-0746, Nihon Kohden). The evoked potential was converted from analog to digital data and stored for subsequent off-line analysis using the PowerLab system running Scope software (version 3; AD Instruments). The intensity of the test stimulation was adjusted for each rat to elicit PSA of approximately 60% of the maximum amplitude, and the PSA in mPFC was determined following the method of previous reports (26, 27) (Fig. 1).

Evoked potential in DG by stimulation of perforant path: A recording electrode was stereotaxically lowered into DG (coordinates: 4.2 mm posterior, 2.5 mm lateral from the bregma, 3.7 mm ventral from the cortical surface), and a bipolar stimulating electrode with a tip separation of 500 μm was placed in the perforant path region (coordinates: 8.0 mm posterior, 4.0 mm lateral from the bregma; 3.0 mm ventral from the cortical surface) under urethane (1 g/kg, i.p.) anesthesia. The recording electrode and stimulating electrode were implanted in the right ipsilateral side. The intensity of the test stimulation was adjusted for each rat to elicit the PSA of approximately 50% of the maximum amplitude. The PSA in DG stimulated by the perforant path (frequency, 0.1 Hz; pulse duration, 250 μs; stimulus interval, 30 s) was obtained from 5 stimuli and was recorded every 2.5 min with a Power Lab Data Acquisition System (AD Instruments) as described above. LTP in DG was induced by tetanic stimulation (Tetanus: main interval, 1 s; 5 trains; each train consisted of 8 pulses at 400 Hz) of the perforant path. LFS (900 pulse at 1 Hz) was given for 15 min prior to LTP-inducing tetanic stimulation. The PSA in DG was determined based on previous reports (7, 28) (Fig. 1).

For determining the evoked potential in DG under consciousness, a recording electrode and a stimulating electrode were inserted into DG and perforant path region, respectively, under pentobarbital (50 mg/kg, i.p.) anesthesia. The electrodes were anchored and affixed to the skull, and five days later, electrophysiological experiments were performed under freely moving conditions following the CFS process. A stimulating electrode was connected through a cable to an electric stimulator with an isolator, and a recording electrode was connected to an amplifier, by similar methods as used for the PSA in mPFC (see above). The intensity of the test stimulation was adjusted for each rat to elicit PSA of approxi-

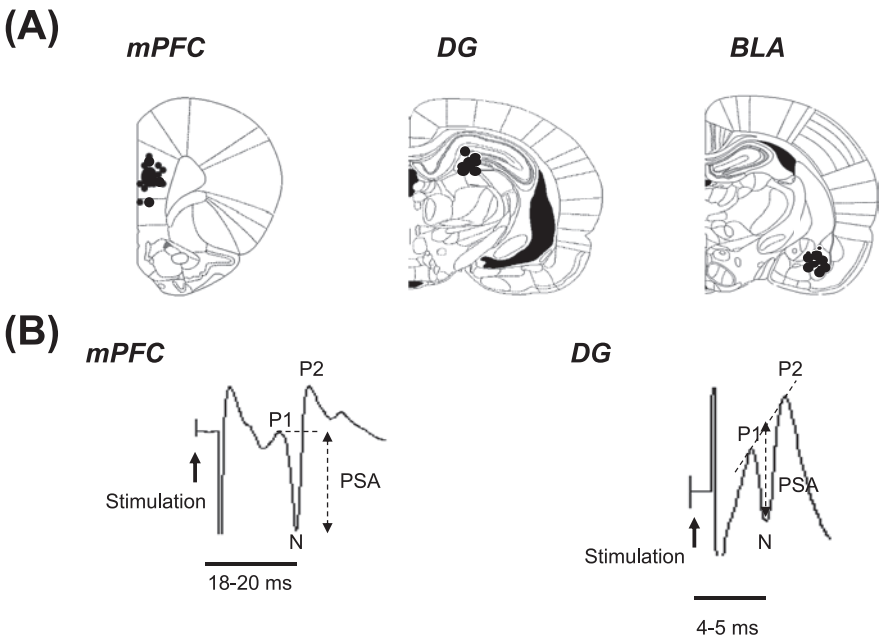

Fig. 1. Diagrams depicting a recording electrode (filled circles) in the prelimbic area of medial prefrontal cortex (mPFC) and the hippocampal dentate gyrus (DG) and a stimulating electrode placement in basolateral amygdala (BLA) (A) and a typical wave-form of an evoked potential in mPFC stimulated by CA1/subicurar region of the ventral hippocampus and in DG stimulated by perforant path (B). The population spike amplitude (PSA) in mPFC was defined as the absolute amplitude of the positive line (P1) and negative peak (N) component of the field potential. PSA in DG was defined as the absolute amplitude between the two dotted lines separating the positive line (between the P1 and P2 components) and the negative (N) peak. PSA is defined as the absolute voltage with a latency of 18 – 20 ms (mPFC) and 4 – 5 ms (DG).

mately 50% of the maximum amplitude. The evoked potential in DG by stimulation of the perforant path (frequency, 0.1 Hz; pulse duration, 250 μs; stimulus interval, 30 s) was obtained from 4 stimuli and was recorded every 2.5 min with a Power Lab Data Acquisition System as described above.

Electrical stimulation of BLA: A bipolar stimulating electrode with a tip separation of 500 μm was placed in the BLA (2.3 mm posterior, 5.0 mm lateral to the bregma, approximately 7.0 mm ventral to the dura). Electrical stimulation of BLA (stimuli intensity, 300 μA; frequency, 0.1 Hz; pulse duration, 250 μs; stimulus interval, 30 s) was initiated 15 min before tetanic stimulation or LFS in the ipsilateral HPC-mPFC pathway or perforant path–DG pathway under urethane (1 g/kg, i.p.) anesthesia. BLA stimulation was completed 15 min prior to tetanus and LFS (i.e., BLA stimulation continued for 15 min and finished prior to tetanus or LFS). In some rats, a recording electrode and a stimulating electrode was inserted into the mPFC and CA1/subcular region, respectively, and a bipolar stimulating electrode was placed in the ipsilateral BLA under pentobarbital (50 mg/kg, i.p.) anesthesia. The electrodes were anchored by small, electrically grounded screws and affixed to the skull with quick, self-curing acrylic resin as described above. Five days later, rats received FS stimuli twice (Day 1 and 2) and subjected to extinction trials three times on day 3. On the day following extinction training, electrophysiological experiments combined with behavioral analysis were performed under freely moving conditions; stimulating electrodes in the CA1/subicular region and BLA were connected through each cable to an electric stimulator (SEN-3301, Nihon Kohden) and isolator (SS-202J or SS-203J, Nihon Kohden). Another cable from the recording electrode in mPFC was connected to an amplifier (MEG-2100 or S-0746, Nihon Kohden). The evoked potential in mPFC was converted from analog to digital data and stored for subsequent off-line analysis using a Power Lab Data Acquisition System as described above. The intensity of the test stimulation was adjusted for each rat to elicit PSA of approximately 60% of the maximum amplitude. Briefly, the synaptic transmission in mPFC was measured in their home cage, and 30 min later, electrical stimulation of BLA (stimuli intensity, 300 μA, frequency, 0.1 Hz; pulse duration, 250 μs; stimulus interval, 30 s) was performed for 15 min in their home cage prior to extinction retrieval. Rats were exposed to the FS box for 10 min as extinction retrieval and then they were returned again into their home cage. The PSA in mPFC was measured throughout the experiments (i.e., pre, during, and post extinction retrieval). Freezing behavior was simultaneously determined during extinction retrieval (10 min). After completion of the electrophysiological experiment, a direct current (900 μA; duration, 20 s) was applied under deep anesthesia, and the brain was removed to histologically verify each electrode placement (Fig. 1).

Experimental protocol

CFS: Adult rats (11 – 14-week-old) were acclimated to the FS box for 5 min and subjected to 5 FS (shock intensity, 1 mA; intershock interval, 28 s; shock duration, 2 s) and then remained further for 5 min without FS stimuli. Twenty-four hours later, rats were re-exposed to the conditioning chamber (FS box) without FS stimuli to verify the retrieval of fear memory. The conditioning FS box [i.e., conditioning chamber; 50 × 16 × 25 cm (height); grid floor (diameter of rods, 0.5 cm; spacing, 1.0 cm)] was composed of opaque acrylic. It should be noted that the conditioning FS box was completely different from the aversive FS box that was used for early postnatal stress (see juvenile stress model). The box was specially constructed by Nihon Kohden for simultaneous determination of electrophysiological and behavioral parameters. Fear-related freezing behavior was defined as lack of movement with the exception of respiration (29). Freezing expression was evaluated by measuring the presence or absence of freezing behavior every 5 s for 5 min immediately after FS conditioning (acquisition) and during exposure to the FS box for 15 min (fear retrieval).

Extinction retrieval of context-dependent fear memory: Extinction protocol was performed in adult rats based on contextual fear conditioning paradigms. The FS conditioning (5 FS: shock intensity, 1 mA; intershock interval, 28 s; shock duration, 2 s) was reinforced by conducting FS stimuli for 2 days (Day 1 and Day 2). Three hours after the second FS stimuli, rats were exposed to the FS box without FS stimuli for 10 min to verify the acquisition of fear memory (acquisition). Twenty-four hours later, rats were re-exposed to the FS box without FS stimuli for 10 min (Extinction trial). This trial was repeated 3 times with 3-h interval between each on day 3 (Extinction training). On the day following extinction training, rats were re-exposed to the FS box for 10 min as extinction retrieval on day 4 (Extinction retrieval). The non-FS control group was divided as follows: No-FS conditioning group [FS (−)], extinction group [Ext (+)], and No-extinction group (i.e., conditioned rats that did not undergo extinction training on day 3) [Ext (−)]. Fear related behavior was evaluated by measuring the presence or absence of freezing behavior every 5 s during extinction retrieval (10 min).

Pharmacological experiment

The *N*-methyl-D-aspartate (NMDA)-receptor antago-

nist MK-801 (0.1 mg/kg; Sigma, St. Louis, MO, USA) or the NMDA-receptor partial agonist D-cycloserine (DCS, 15 mg/kg; Sigma) was dissolved in saline and injected intraperitoneally (i.p.) 20 min before LFS application prior to tetanus in the Non-FS group (control) and 3wFS group, respectively. The injection route and dosage of MK-801 and DCS were chosen based on previous reports (18) and (24), respectively.

Statistical analyses

Data are expressed as the mean ± standard error of the mean (S.E.M.). Electrophysiological data are expressed as a percentage of baseline before tetanus, exposure to CFS, LFS or tetanus. The area under the curve (AUC) (%·min) was used to evaluate the PSA ensemble effects and was calculated after tetanus (for 60 min). In the case of the extinction protocol, data are expressed as a percentage of baseline before extinction retrieval (exposure to FS box) and the AUC was calculated after extinction retrieval for 60 min. AUC data were analyzed using one-factor analysis of variance (ANOVA) followed by Scheffe's test for multiple comparisons. The unpaired Student's *t*-test was also used to compare data. Freezing behavior during extinction retrieval is expressed as the percentage of counts determined in 10-min blocks. Values of $P < 0.05$ were considered statistically significant.

Results

Characteristics of synaptic metaplasticity in the HPC-mPFC pathway, compared with those in the hippocampal DG

The synaptic metaplasticity was characterized by elucidating the effects of BLA activation or stress exposure on LTP in mPFC and DG. As shown in Fig. 2A-1, prior BLA activation did not cause any changes in the basal synaptic transmission or tetanus-induced LTP induction in mPFC under anesthesia (Fig. 2A-1). BLA activation did not alter the basal synaptic transmission but enhanced the magnitude of LTP in DG (Fig. 2B-1). In order to elucidate the effects of stress exposure on LTP induction, tetanus was applied after CFS under consciousness. The synaptic transmission in mPFC was slightly reduced during exposure to CFS, but LTP was not affected by CFS (Fig. 2A-2). In contrast, the magnitude of LTP in DG was significantly enhanced by CFS (Fig. 2B-2). We next examined the effects of LFS prior to tetanus on subsequent LTP, focusing on the electrophysiological profile of synaptic metaplasticity. LTP induction in mPFC was not affected by LFS prior to tetanus (Fig. 3A), whereas the magnitude of LTP in DG was enhanced by LFS (Fig. 3B). These data show that synaptic changes induced by CFS or BLA activation in mPFC and DG were similar to those induced by LFS prior to tetanus.

Possible involvement of BLA modulation of synaptic metaplasticity in the HPC-mPFC pathway, compared with those in the hippocampal DG

On the basis of previous findings that synaptic metaplasticity in the CA1 field is regulated by BLA (19, 20), we elucidated whether BLA modulation is involved in the synaptic changes induced by LFS prior to tetanus under anesthesia. As shown in Fig. 3A, LFS-primed LTP in the mPFC was markedly suppressed by prior BLA activation. ANOVA ($F_{2,16} = 11.325$, $P < 0.01$) followed by Scheffe's *post-hoc* testing revealed significant decreases in AUC of PSA for the BLA-stimulated group compared with the non-stimulated control ($P < 0.05$). BLA stimulation throughout the experiment (i.e., pre, during, and post LFS) (AUC, %·min/10^3; 5.23 ± 0.73, n = 4) tended to augment the synaptic suppression compared with those by prior BLA activation (6.18 ± 0.32, n = 6). These data suggest that the LFS-primed LTP in mPFC was regulated by BLA in an inhibitory manner. Prior BLA activation did not significantly influence the LFS-primed LTP augmentation in DG (Fig. 3B), indicating the possibility that the enhancement of LTP induced by LFS exhibits ceiling effects.

Possible involvement of BLA modulation of synaptic potentiation in the HPC-mPFC pathway associated with extinction retrieval

To investigate the significant role of the modulatory effects of BLA, we focused on the synaptic function in mPFC associated with fear extinction under consciousness, compared with those in 3wFS that exhibited extinction deficit (23, 24). The synaptic transmission in mPFC was slightly decreased during extinction retrieval, but synaptic efficacy was subsequently enhanced, accompanied with decreases in freezing behavior. As shown in Fig. 4, A and B, prior BLA activation significantly prevented the synaptic potentiation, i.e., LTP-like response and attenuated decreases in freezing behavior responsible for extinction retrieval. ANOVA ($F_{3,25} = 16.641$, $P < 0.001$) followed by Scheffe's *post-hoc* testing revealed significant decreases in AUC of PSA in the BLA-stimulated group compared with the non-stimulated control ($P < 0.05$). The BLA-mediated synaptic inhibition and behavioral changes are similar to those observed in the 3wFS group; synaptic potentiation responsible for extinction retrieval did not occur in the 3wFS group (Fig. 4A). ANOVA ($F_{4,31} = 19.166$, $P < 0.001$) followed by Scheffe's *post-hoc* testing revealed significant differences in freezing behavior between the 3wFS group, as well as the BLA-stimulated group, and non-stimulated

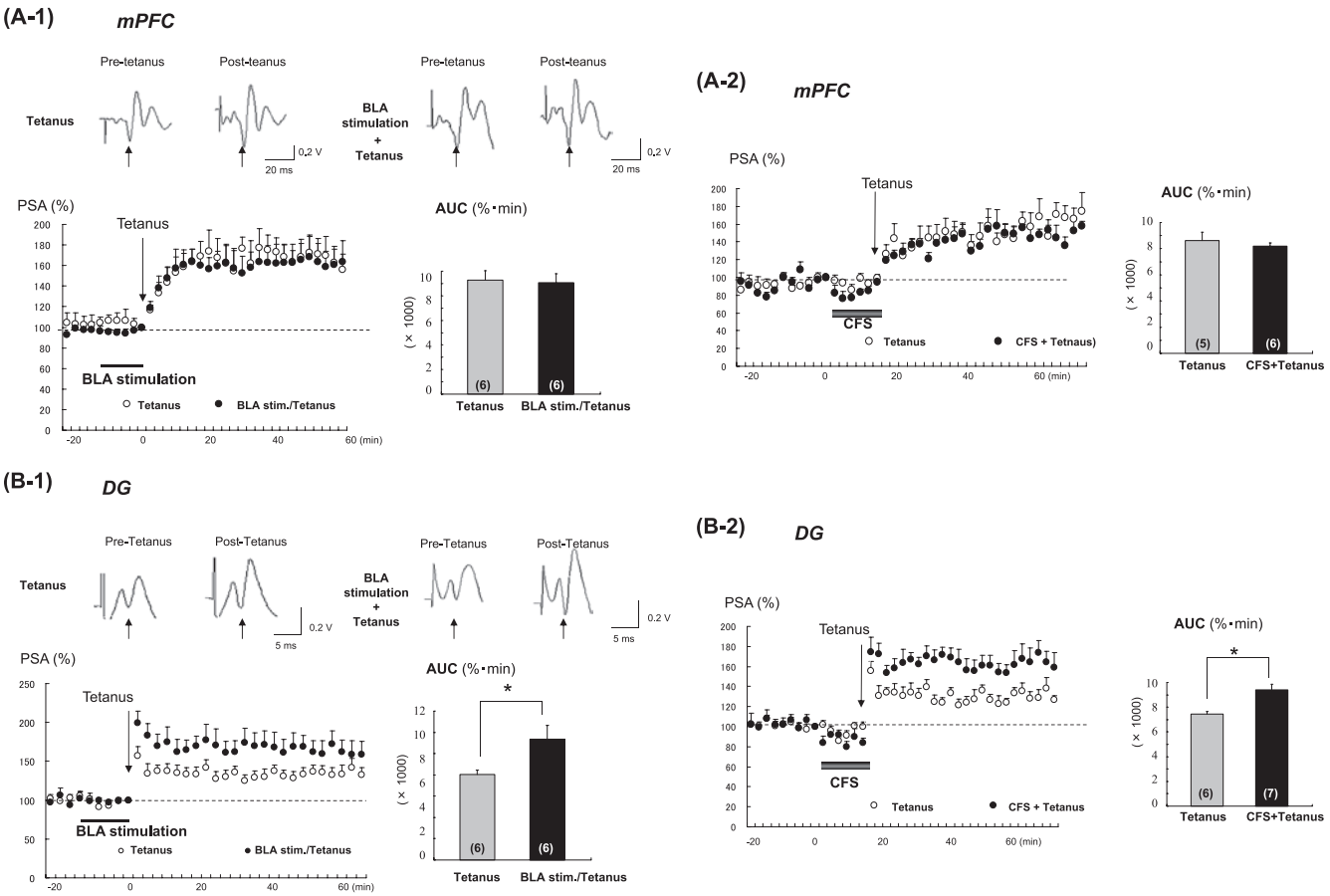

Fig. 2. Effects of basolateral amygdala (BLA) activation or exposure to conditioned fear stress (CFS) on long-term potentiation (LTP) in medial prefrontal cortex (mPFC) (A-1 and A-2) and hippocampal dentate gyrus (DG) (B-1 and B-2). Specimen recordings and time-course response of the population spike amplitude (PSA) induced by high-frequency stimulation (Tetanus) after BLA stimulation in the mPFC (A-1) and DG (B-1). Specimen recordings showed changes in PSA before (pre) and after (post) tetanus with or without BLA activation. The arrow represents PSA with a latency of 18 – 20 ms (mPFC) and 4 – 5 ms (DG). Each time-course response is presented as the percentage of changes in PSA. Area under the curve values (AUC, %·min/10^3) are presented as the percentage of changes in PSA and integrated PSA for 60 min after tetanus. The number of animals used is indicated within each column. Each value represents the mean ± S.E.M. *$P < 0.05$.

control ($P < 0.05$). There were no significant differences in freezing expression in the non-extinction group [Ext (−)] with or without BLA stimulation (Fig. 4B). BLA stimulation also did not alter freezing in the non-conditioned group (data not shown). Thus, BLA stimulation by itself did not influence the freezing expression. As shown in Fig. 4C, the synaptic response evaluated by AUC inversely correlated with freezing expression observed during extinction retrieval ($P < 0.01$). Thus, synaptic changes and the behavioral alteration induced by BLA activation were mimicked by those observed in the 3wFS group. These results lead us to suppose that synaptic dysfunction associated with extinction deficit in the 3wFS group may be involved in the BLA modulation.

Characteristics of LFS-primed LTP in the HPC-mPFC pathway, compared with those in the 3wFS group

Based on this speculation, we elucidated pharmacologically the characteristics of LFS-primed LTP in mPFC, focusing on the NMDA-receptor–mediated mechanisms. Consistent with a previous report (18), the NMDA-receptor antagonist MK-801 (0.1 mg/kg, i.p.) by itself did not affect the LTP induction (data not shown). However, pretreatment with MK-801 (0.1 mg/kg, i.p.) completely prevented the LFS-primed LTP in mPFC (Fig. 5A). These data indicate that NMDA-mediated mechanisms are involved in the LFS-primed LTP in mPFC, as well as homosynaptic metaplasticity in the CA1 field (30, 31). In the 3wFS group, LTP was not induced but was obviously suppressed by LFS prior to tetanus (Fig. 5B). ANOVA ($F_{2,15} = 7.554$, $P < 0.001$)

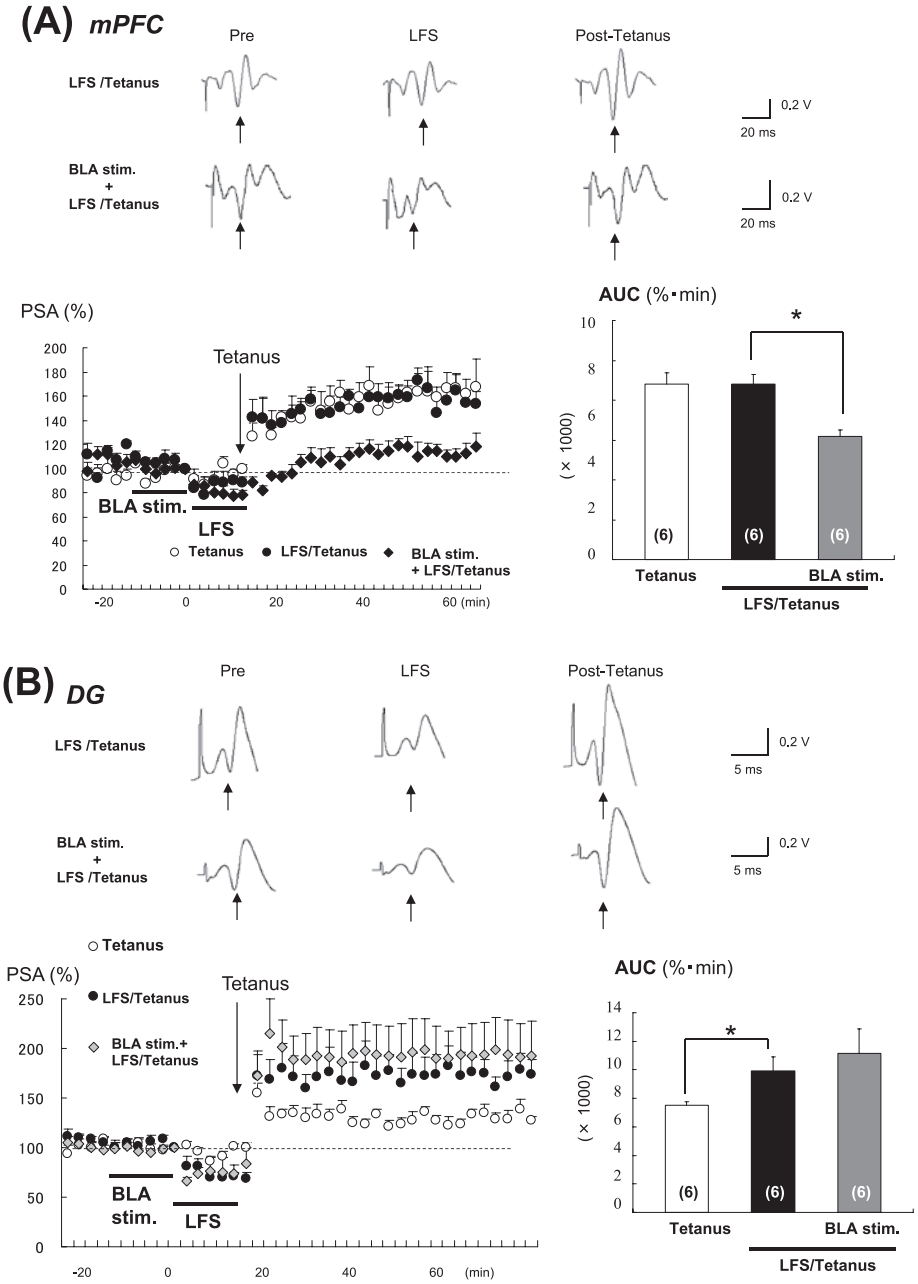

Fig. 3. Changes in the long-term potentiation (LTP) induced by low-frequency stimulation (LFS) prior to high-frequency stimulation (Tetanus) in the medial prefrontal cortex (mPFC) (A) and hippocampal dentate gyrus (DG) (B) with or without basolateral amygdala (BLA) activation. LFS (1 Hz) was applied for 15 min prior to tetanus. BLA stimulation was initiated 15 min before LFS and was completed 15 min prior to tetanus. Specimen recordings show changes in population spike amplitude (PSA) before (pre) and during LFS and after (post) tetanus with or without BLA stimulation. Arrow represents PSA with a latency of 18 – 20 ms (mPFC) and 4 – 5 ms (DG). Time-course response is presented as the percentage of changes in the PSA. Area under the curve values (AUC; %·min/10^3) are presented as the percentage of changes in PSA and integrated PSA for 60 min after tetanus. The number of animals used is indicated within each column. Each value represents the mean ± S.E.M. *$P < 0.05$.

followed by Scheffe's *post-hoc* testing revealed significant differences in AUC of PSA for the LFS-primed 3wFS group compared with the non-primed group (Tetanus, $P < 0.05$). This synaptic inhibition was similar to that induced by prior BLA activation (Fig. 3A). Furthermore, pretreatment with the NMDA-receptor partial agonist DCS (15 mg/kg, i.p.) prevented the LTP suppression induced by LFS applied to tetanus in the 3wFS group; no significant difference was observed between the DCS-treated group (LFS-primed group) and non-primed group (Tetanus) (Fig. 5B). Thus, LFS-primed LTP suppression in the 3wFS group tended to be recovered by pretreatment with DCS. These results suggest that BLA modulation contributes to the synaptic dysfunction observed in the 3wFS group, in which NMDA-receptor–mediated mechanisms are involved.

Discussion

Several lines of evidence showed that the hippocampal subregions have differential susceptibility to BLA activation or stress exposure (5, 7). In this study, the magnitude of LTP in the DG was enhanced by either BLA activation or CFS. Given the evidence that LTP in the

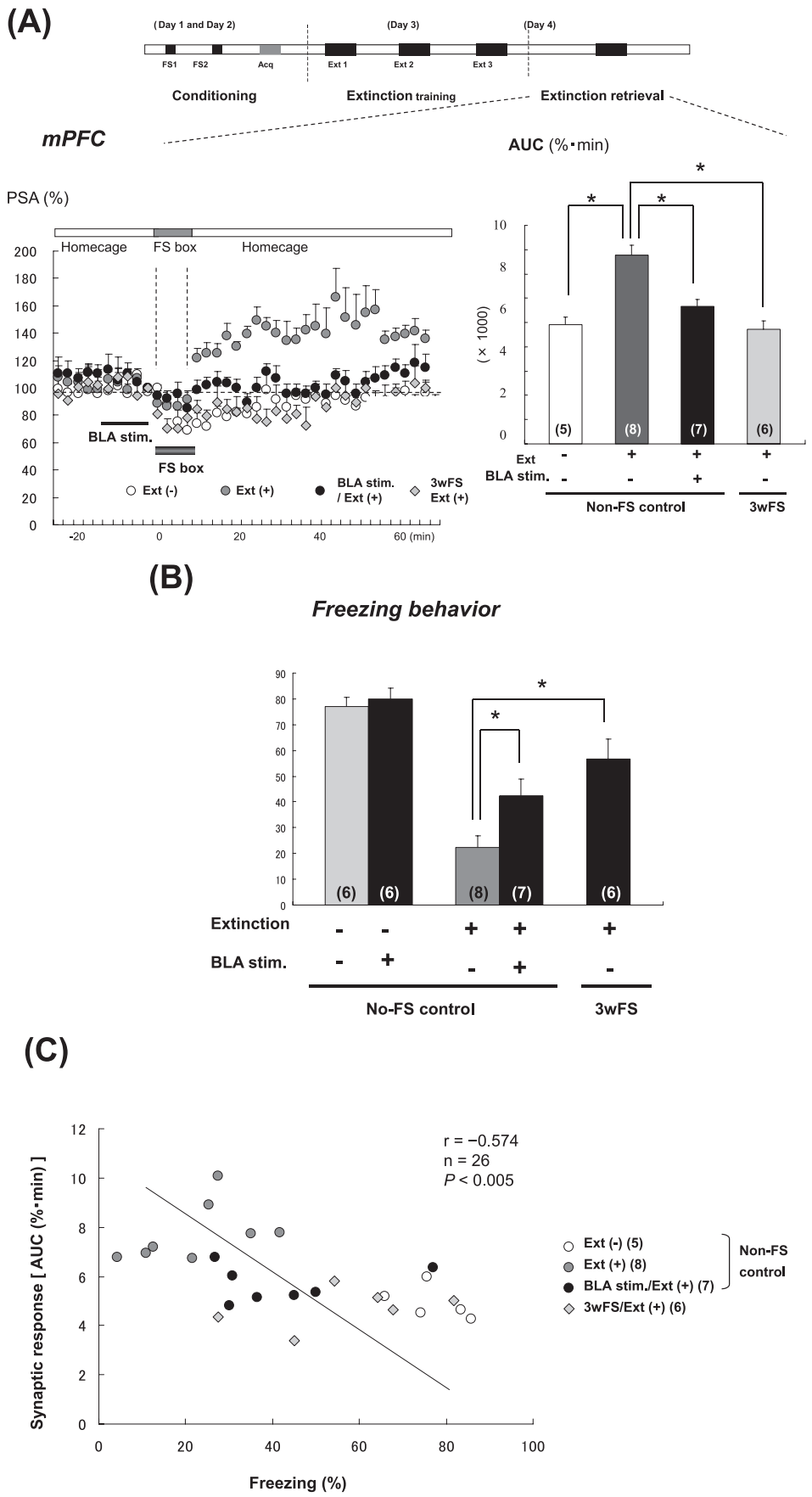

Fig. 4. Effects of basolateral amygdala (BLA) activation on synaptic response in medial prefrontal cortex (mPFC) (A) and behavioral response (B) during extinction retrieval based on contextual fear conditioning paradigm under freely moving conditions, and their correlation (C). Rats received footstock (FS) stimuli twice (Days 1 and 2) and were re-exposed to the context (FS box) without FS stimuli. This extinction trial was repeated 3 times at 3-h intervals (extinction training, Day 3). On the day following extinction training, rats were re-exposed to the same context (FS box) as extinction retrieval (Day 4). Ext (−): conditioned group that did not undergo extinction training (i.e., rats remained in their homecage on Day 3 instead of extinction training) and Ext (+): conditioned group exposed to extinction trials. Non-FS control: adult rats that did not receive early postnatal stress. 3wFS: adult rats that received aversive FS stimuli during the third postnatal period. BLA stimulation was applied for 15 min in their home cage, and then rats were re-exposed to the context (FS box) as extinction retrieval for 10 min. A) Time-course of synaptic response in mPFC presented as the percentage of changes in PSA before extinction retrieval. Area under the curve values (AUC, %·min/10^3) are presented as the percentage of changes in the PSA and integrated PSA for 60 min after extinction retrieval. The number of animals used is indicated within each column. Each value represents the mean ± S.E.M. *$P < 0.05$. B) Freezing expression in Non-FS control and 3wFS group with [Ext (+)] or without [Ext (−)] extinction training. BLA stimulation was initiated 15 min before exposure to FS box (i.e., extinction retrieval) and was completed 15 min prior to extinction retrieval in both Ext (−) group and Ext (+) group. In no-extinction group [Ext (−)], BLA stimulation was carried out 15 min before exposure to another context box. The presence or absence of freezing behavior was estimated every 5 s and was expressed as the percentage of total freezing for 10 min. Values are expressed as the mean ± S.E.M. The numbers of rats tested are shown within each column. *$P < 0.05$. C) Correlation between the expression of freezing during extinction retrieval and the synaptic response in mPFC. AUC (%·min/10^3) are presented as the percentage of changes in the PSA and integrated PSA for 60 min after extinction retrieval. The numbers of rats tested are shown in parentheses. Freezing behavior in each rat during extinction retrieval inversely correlated with the synaptic response evaluated by AUC of the PSA ($P < 0.01$).

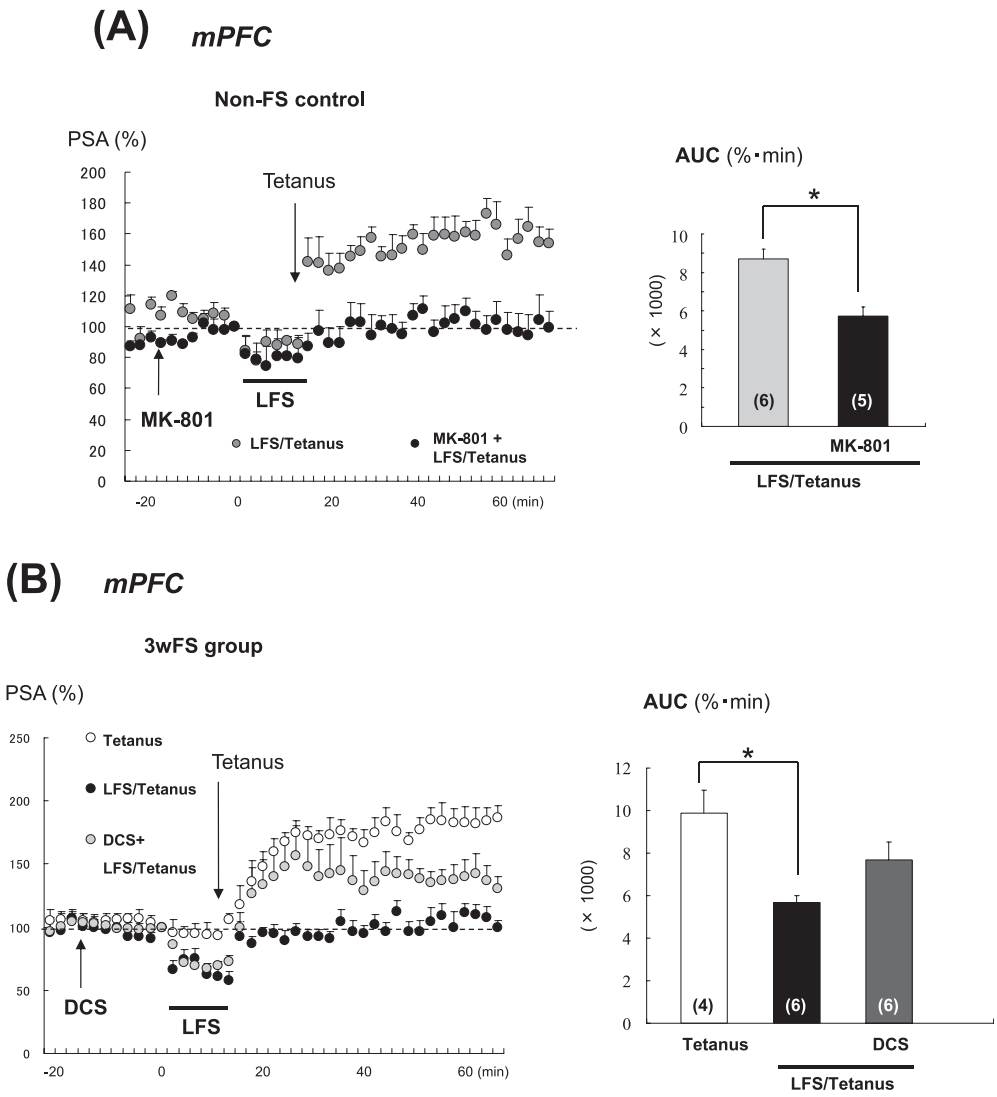

Fig. 5. Effects of NMDA receptor antagonist MK-801 in Non-FS control (A) and NMDA receptor agonist D-cycloserine in 3wFS group (B) on LFS-primed long-term potentiation (LTP) in medial prefrontal cortex (mPFC). MK-801 (0.1 mg/kg, i.p.) and D-cycloserine (DCS, 15 mg/kg, i.p.) were administered 20 min before application of low frequency stimulation (LFS). LFS (1 Hz) was applied for 15 min prior to tetanus and was completed 15 min prior to tetanus. 3wFS group: adult rats that received aversive footshock (FS) stress during the third postnatal period. Time-course response is presented as the percentage of changes in the population spike amplitude (PSA). Area under the curve values (AUC, % · min/10^3) are presented as the percentage of changes in PSA and integrated PSA for 60 min after tetanus. The number of animals used is indicated within each column. Each value represents the mean ± S.E.M. *$P < 0.05$.

CA1 field was impaired by BLA activation (7, 32) and CFS (33, 34), the present data strengthen the findings that stress exposure, in addition to BLA activation, has opposing effects on synaptic plasticity in a hippocampal subregion–specific manner. These synaptic changes mimicked those induced by LFS prior to tetanus; LFS application prior to tetanus caused enhancement of LTP in DG, whereas it suppressed LTP in the CA1 field (20). Synaptic metaplasticity in the CA1 field (i.e., LTP suppression by LFS application prior to tetanus) is considered to serve as a cellular mechanism of stress experience–dependent fear memory (20, 31, 35). Therefore, LFS-primed synaptic changes in the hippocampal subregions appear to reflect one aspect of responses to CFS. In contrast to the hippocampus, neither BLA activation nor CFS had any effect on the magnitude of LTP in mPFC. LFS application prior to tetanus also did not affect LTP in mPFC. In other words, the BLA appears to impact on the hippocampal synaptic changes associated with fear conditioning.

An important finding is that prior BLA activation suppressed the LFS-primed LTP in mPFC. These results suggest that BLA activation can alter the threshold for LFS, affecting the maximal level of LTP, which results in LTP suppression. This BLA-mediated synaptic response seems to be similar to the synaptic changes observed in extinction processes following BLA activation; prior BLA activation suppressed the synaptic potentiation responsible for extinction retrieval and attenuated decreases in fear-related freezing behavior. Although the occurrence and strength of LTP in the HPC-mPFC pathway is considered to reflect synaptic plasticity in the mPFC (36), we cannot completely exclude the possibility that the BLA-mediated synaptic response in the mPFC might result from indirect interaction between the BLA and ventral hippocampus (CA1/

subicular region). Given the role of the hippocampus in contextual encoding (37), it is plausible that the hippocampus plays an important role in contextual processing of fear extinction. However, we have previously shown that region-specific synaptic changes occurred in the extinction processes; differing from synaptic changes in the mPFC, synaptic transmission in the CA1 field did not cause any changes after extinction retrieval (38). Therefore, it seems unlikely that the BLA directly modulated hippocampal function and consequently lead to suppression of the synaptic potentiation in the mPFC, at least in the extinction retrieval. Taken together, these results strongly suggest that the BLA can modulate cortical synaptic function associated with extinction retrieval.

Interestingly, extinction retrieval can be accompanied by conversion from acute depression to long-term enhancement of synaptic efficacy in the mPFC, which resembles the synaptic pattern following LFS prior to tetanus. Such bidirectional phenomena (i.e., transient synaptic depression and subsequent synaptic potentiation) seem to be attributable to the functional specificity associated with extinction retrieval. It is generally accepted that extinction does not erase the original fear memory, but yields a new inhibitory memory, named extinction memory, that reduces fear to conditioned stimulus (15, 39). It is plausible, therefore, that original fear memory and extinction memory compete with each other during extinction retrieval. In turn, the bidirectional synaptic pattern may reflect labile states of original fear memory and extinction memory. Herry and Garcia (40) reported that synaptic depression induced by tone-dependent extinction retrieval did not interfere with extinction but predicted spontaneous recovery of the fear response. Indeed, we observed that decreases in the synaptic transmission in the mPFC occurred during fear retrieval (i.e., exposure to CFS). Speculatively, synaptic depression observed during extinction retrieval may be related to the fear response induced by context-cued stimulus, which may alter the thresholds at the synaptic transmission that affect the subsequent synaptic efficacy. This hypothesis was supported by the data observed in the 3wFS group that exhibited extinction deficit: synaptic transmission was markedly decreased during extinction retrieval, and subsequent synaptic potentiation did not occur. The synaptic changes and behavioral alteration in the 3wFS group exhibited a similar phenomenon during extinction retrieval following prior BLA activation. Furthermore, LTP was not induced, but suppressed by LFS prior to tetanus in the 3wFS group. This synaptic response was mimicked by the synaptic change by BLA activation (i.e., prior BLA activation prevented the subsequent LTP by LFS application). These data suggest that cortical synaptic dysfunction observed in the 3wFS group was caused by BLA activation. In other words, LFS-primed LTP in the mPFC appears to be associated with the neural bases of fear extinction.

In this study, blocking the NMDA receptors by MK-801 completely prevented the LFS-primed LTP in the mPFC, indicating the involvement of NMDA-mediated mechanisms in the synaptic response, as well as LTP induction in the mPFC (26) and LFS-induced metaplasticity in the CA1 field (30, 31). In contrast, LTP suppression following LFS in the 3wFS group was reversed by pretreatment with the NMDA-receptor partial agonist DCS. NMDA receptors have been strongly implicated in mechanisms underlying learning and memory, including extinction memory (41, 42). Indeed, we observed that cortical synaptic dysfunction and behavioral deficit in the 3wFS group were ameliorated by systemic administration of DCS (24). Although the precise target site of DCS is unclear because of its systemic administration, several studies have shown that intra-BLA infusion of DCS facilitates fear extinction (43) and that intra-BLA injection of the selective NMDA-receptor antagonist AP-5 prevents fear extinction (44, 45). Considering the similarity of the synaptic response and behavioral changes following prior BLA activation, synaptic dysfunction and extinction deficit in the 3wFS group might result from hyperactivity of the BLA, in which NMDA-receptor–mediated mechanisms are involved.

It should be noted that the present results appear to be inconsistent with previous reports stating that BLA activation and/or elevated platform stress suppressed LTP in the mPFC (18, 46). Although we cannot adequately explain these controversial results, one plausible explanation may be the differential parameters such as variation or intensity of BLA stimulation used in this study. Li and Richter-Levin (47) recently reported the existence of biphasic modulation of the BLA on LTP in the DG; the higher intensity of BLA stimulation (2V) did not enhance but instead impaired LTP in the DG. These findings lead us to suggest that LTP in the mPFC may be influenced by the degree of BLA activation. Another explanation may arise from the different characteristics or timing of stress paradigms. Namely, Richter-Levin et al. (18) studied in rats which were exposed to innate fear stress by exposure to an elevated platform and were immediately anesthetized for electrophysiological experiments. On the other hand, in the present study, CFS based on fear memory was performed 24 h after FS conditioning, and PSA was determined under freely moving conditions. Although further studies are required, CFS did not influence LTP in the mPFC, at least under the present experimental conditions, despite enhance-

ment and impairment of LTP in the DG and CA1, respectively. These findings are supported in part by our previous biochemical evidence in which CFS suppressed the activation of extracellular signal–regulated kinase, a critical intracellular cascade implicated in memory formation in the CA1 field, but not in the mPFC (48).

Conclusion

To the best of our knowledge, this is the first demonstration of the physiological interaction between BLA modulation and cortical synaptic function in the extinction processes. Prior BLA activation suppressed synaptic potentiation in the HPC-mPFC pathway and attenuated decreases in freezing behavior responsible for extinction retrieval. BLA activation also suppressed LFS-primed LTP prior to tetanus in the mPFC. These synaptic changes were similar to those observed in the 3wFS group that exhibited extinction deficit. In other words, synaptic dysfunction in the 3wFS group might be caused by exaggerated BLA activity resulting from exposure to early postnatal stress. A better understanding of region-specific alterations of synaptic function in the HPC-mPFC pathway with dynamic interactions in BLA networks may contribute to the study of the pathophysiology of anxiety disorders including post-traumatic disorder, thereby leading to the development of novel therapeutic strategies.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research to H.T. (No. 24590117) from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan and a Grant-in-Aid for the 2012-2013 Research Project of the Research Institute of Personalized Health Sciences, Health Sciences University of Hokkaido, Japan.

References

1 Abraham WC, Bear NF. Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci. 1996;19:126–130.
2 Abraham WC. Metaplasticity: tuning synapses and networks for plasticity. Nature Reviews Neurosci. 2008;9:387–399.
3 Shors TG, Gallegos RA, Breindl A. Transient and persistent consequences of acute stress on long-term potentiation (LTP), synaptic efficacy, theta rhythms and bursts in area CA1 of the hippocampus. Synapse. 1997;26:209–217.
4 Shakesby AC, Anwyl R, Rowan MJ. Overcoming the effects of stress on synaptic plasticity in the intact hippocampus: rapid actions of serotonergic and antidepressant agents. J Neurosci. 2002;22:3638–3644.
5 Kavushansky A, Vouimba R-M, Cohen H, Richter-Levin G. Activity and plasticity in the CA1, the dentate gyrus, and the amygdala following controllable vs. uncontrollable water stress. Hippocampus. 2006;16:35–42.
6 Spyrka J, Hess G. Repeated restraint-induced modulation of long-term potentiation in the dentate gyrus of the mouse. Brain Res. 2010;1320:28–33.
7 Vouimba R-M, Richter-Levin G. Physiological dissociation in hippocampal subregions in response to amygdala stimulation. Cereb Cortex. 2005;15:1815–1821.
8 Frey S, Bergado-Rosado J, Seidenbecher T, Pape HC, Frey JU. Reinforcement of early long-term potentiation (early-LTP) in dentate gyrus by stimulation of the basolateral amygdala: heterosynaptic induction mechanisms of late-LTP. J Neurosci. 2001;21:3697–3703.
9 Vouimba R-M, Yaniv D, Diamond D, Richter-Levin G. Effects of inescapable stress on LTP in the amygdala versus the dentate gyrus of freely behaving rats. Eur J Neurosci. 2004;19:1887–1894.
10 Kim JJ, Lee HJ, Han J-S, Packard MG. Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. J Neurosci. 2001;21:5222–5228.
11 Pikkarainen M, Ronkko S, Savander V, Insausti R, Pitkanen A. Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. J Comp Neurol. 1999;403:229–260.
12 Bacon SJ, Headlam AJN, Gabbott PLA, Smith AD. Amygdala input to medial prefrontal cortex (mPFC) in the rat: a light and electron microscope study. Brain Res. 1996;720:211–219.
13 Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum and prefrontal cortex. Neurosci Biobehav Rev. 2002;26:321–352.
14 Maren S, Quirk GJ. Neuronal signalling of fear memory. Nat Rev Neurosci. 2002;5:844–852.
15 Myers KM, Davis M. Mechanisms of fear extinction. Mol Psychiatry. 2007;12:120–150.
16 Quirk GJ, Mueller D. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology. 2008;33:56–72.
17 Godsil BP, Kiss JP, Spedding M, Jay TM. The hipppocampal-prefrontal pathway: the weak link in psychiatric disorders? Eur Neuropsychopharmacol. 2013;23:1165–1181.
18 Richter-Levin G, Maroun M. Stress and amygdala suppression of metaplasticity in the medial prefrontal cortex. Cereb Cortex. 2010;20:2433–2441.
19 Kim JJ, Koo JW, Lee HJ, Han J-S. Amygdalar inactivation blocks stress-induced impairments in hippocampal long-term potentiation and spatial memory. J Neurosci. 2005;35:1532–1539.
20 Hirata R, Matsumoto M, Judo C, Yamaguchi T, Izumi T, Yoshioka M, et al. Possible relationship between the stress-induced synaptic response and metaplasticity in the hippocampal CA1 field of freely moving rats. Synapse. 2009;63:549–556.
21 Ziabreva I, Poeggel G, Schnabel R, Braun K. Separaion-induced receptorchanges in the hippocampus and amygdala of octodon degus:influence f maternal vocalizations. J Neurosci. 2003;23:5329–5366.
22 Blaise JH, Koranda JL, Chow U, Haines KE, Dorward EC. Neonatal isolation stress alters bidirectional long-term synaptic plasticity in amygdalo-hippocampal synapses in freely behaving adult rats. Brain Res. 2008;1193:25–33.
23 Matsumoto M, Togashi H, Konno K, Koseki H, Hirata R, Izumi T, et al. Early postnatal stress alters the extinction of context-dependent conditioned fear in adult rats. Pharmacol Biochem Behav. 2008;89:247–252.
24 Judo C, Matsumoto M, Yamazaki D, Hiraide S, Yanagawa Y,

Kimura S, et al. Early stress exposure impairs synaptic potentiation in the rat medial prefrontal cortex underlying contextual fear extinction. Neuroscience. 2010;169:1705–1714.
25 Koseki H, Matsumoto M, Togashi H, Miura Y, Fukushima K, Yoshioka M. Alteration of synaptic transmission in the hippocampal-mPFC pathway during extinction trials of context-dependent fear memory in juvenile rat stress models. Synapse. 2009;63:805–813.
26 Jay TM, Burette F, Laroche S. NMDA receptor-dependent long-term potentiation in the hippocampal afferent fibre system to the prefrontal cortex in the rat. Eur J Neurosci. 1995; 7:247–250.
27 Hugus S, Garcia R. Reorganization of learning-associated prefrontal synaptic plasticity between the recall of recent and remote fear extinction memory. Learn Mem. 2007;14;520–524.
28 Naie K, Manahan-Vaughan D. Regulation by metabotropic glutamate receptor 5 of LTP in the dentate gyrus of freely moving rats: relevance for learning and memory formation. Cereb Cortex. 2004;14:189–198.
29 Fanselow MS. Conditioned and unconditional components of post-shock freezing. Pavlov J Biol Sci. 1980;15:177–182.
30 Mockett B, Coussens C, Abraham WC. NMDA receptor-mediated metaplasticity during the induction of long-term depression by low-frequency stimulation. Eur J Neurosci. 2002; 15:1819–1826.
31 MacDonald JF, Jackson MF, Beazely MA. G protein-coupled receptors control NMDARs and metaplasticity in the hippocampus. Biochim Biophys Acta. 2007;1768:941–951.
32 Vouimba R-M, Richter-Levin G. Different patterns of amygdala priming differentially affect dentate gyrus plasticity and corticosterone, but not CA1 plasticity. Front Neural Circuits. 2013; 7:1–10.
33 Matsumoto M, Tachibana K, Togashi H, Tahara K, Kojima T, Yamaguchi T, et al. Chronic treatment with milnacipran reverses the impairment of synaptic plasticity induced by conditioned fear stress. Psychopharmacology. 2005;179:606–612.
34 Hirata R, Togashi H, Matsumoto M, Yamaguchi T, Izumi T, Yoshioka M. Characterization of stress-induced suppression of long-term potentiation in the hippocampal CA1 field of freely moving rats. Brain Res. 2008;1226:27–32.
35 Zelcer I, Cohen H, Richter-Levin G, Lebiosn T, Grossberger T, Barkai E. Cellular correlate of learning-induced metaplasticity in the hippocampus. Cereb Cortex. 2006;16:460–468.
36 Jay TM, Thierry AM, Wiklund L, Glowinski J. Excitatory amino-acid pathway from the hippocampus to the prefrontal cortex. Contribution of AMPA receptors in hippocampo-prefrontal cortex transmission. Eur J Neurosci. 1992;4:1285–1295.
37 Ji J, Maren S. Hippocampal involvement in contextual modulation of fear extinction. Hippocamppus. 2007;17:749–758.
38 Saito Y, Matsumoto M, Otani S, Yanagawa Y, Sachiko Hiraide S, Ishikawa S, et al. Phase-dependent synaptic changes in the hippocampal CA1 field underlying extinction processes in freely moving rats. Neurobiol Learn Mem. 2012;97:361–369.
39 Quirk GJ, Mueller D. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology. 2008;33:56–72.
40 Herry C, Garcia R. Prefrontal cortex long-term potentiation, but not long-term depression, is associated with the maintenance of extinction of learned fear in mice. J Neurosci. 2002;22:577–583.
41 Richardson R, Ledgerwood L, Cranney J. Facilitation of fear extinction by D-cycloserine: theoretical and clinical implications. Learn Mem. 2004;11:510–516.
42 Davis M, Ressler K, Rothbaum BO, Richardson R. Effects of D-cycloserine on extinction: translation from preclinical to clinical work. Biol Psychiatry. 2006;60:369–375.
43 Mao S-C, Hsiao Y-H, Gean P-W. Extinction training in conjunction with a partial agonist of the glycine site on the NMDA receptor erases memory trace. J Neurosci. 2006;26:8892–8899.
44 Falls WA, Miserendino MJ, Davis M. Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. J. Neurosci. 1992;12:854–863.
45 Zimmwemn JM, Maren S. NMDA receptor antagonism in the basolateral but not central amygdale blocks the extinction of Pavlovian fear conditioning in rats. Eur J Neurosci. 2010;31: 1664–1670.
46 Rocher C, Spedding M, Munoz C, Jay TM. Acute stress-induced changes in hippocampal prefrontal circuits in rats: effects of antidepressants. Cereb Cortex. 2004;14:224–229.
47 Li Z, Richter-Levin G. Stimulus intensity-dependent modulations of hippocampal ong-term potentiation by basolaeral amygdale priming. Front Cell Neurosci. 2012;6:1–9.
48 Ishikawa S, Saito Y, Yanagawa Y, Otani S, Hiraide S, Shimamura K, et al. Early postnatal stress alters extracellular signal-regulated kinase signaling in the corticolimbic system modulating emotional circuitry in adult rat. Eur J Neurosci. 2012;35:135–145.