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Possible Modulation of the Amygdala on Metaplasticity Deficits in the Hippocampal CA1 Field in Early Postnatally Stressed Rats

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Abstract. Several lines of evidence have shown that early life experiences have a profound impact on fear-related behavior, but the detailed mechanisms are unknown. The present study examined the possible involvement of the amygdala in behavioral deficits associated with fear memory in a juvenile stress model, with a focus on hippocampal synaptic function. Adult rats exposed to footshock (FS) stress during the second postnatal period (2wFS group) exhibited low levels of freezing in response to contextual fear conditioning (CFC). The CFC-induced suppression of long-term potentiation (LTP) in the CA1 field was not found in the 2wFS group. Additionally, synaptic metaplasticity, that is, low-frequency stimulation-induced suppression of subsequent LTP, did not occur in the 2wFS group; instead, LTP was induced. These synaptic changes mimicked the impairment in metaplasticity induced by reversible inactivation of the basolateral amygdala (BLA). Inactivation of the BLA markedly decreased freezing behavior in non-FS controls, similar to the 2wFS group. Furthermore, extracellular signal-regulated kinase activation in the BLA in response to CFC did not occur in the 2wFS group. These findings suggest that early postnatal stress may cause long-term dysfunction of the modulatory effect of the amygdala on hippocampal function associated with fear memory.

Keywords: early postnatal stress, metaplasticity, hippocampal CA1 field, amygdala modulation, contextual fear conditioning

Introduction

The hippocampus is known to be a key structure responsible for the contextual modulation of fear memory (1, 2). Synaptic transmission in the hippocampal CA1 field was reduced during the retention session of a contextual fear conditioning (CFC) paradigm (3, 4). Synaptic plasticity, reflected by long-term potentiation (LTP), was also suppressed after the CFC retention session (5, 6), suggesting that stressful experiences caused synaptic inhibition, resulting in the suppression of LTP. The suppression of LTP in the CA1 field was caused not only by stress paradigms but also by low-frequency stimulation (LFS) prior to LTP-inducing high frequency stimulation (tetanus), termed metaplasticity (7, 8). This homosynaptic metaplasticity appears to reflect one aspect of hippocampal function in the mediation of responses to stress because of the similar electrophysiological (9, 10) and neurochemical (11) profiles.

Hippocampal synaptic function is also heterosynaptically modulated by the amygdala, a key structure involved in the conditioned fear response (12, 13). For example, prior amygdala activation suppressed the induction of LTP in the CA1 field (14). Modulation by the amygdala also affects the synaptic response to a subsequent emotional event. Electrolytic lesions or pharmacological inactivation of the amygdala suppressed the effects of "stress" on LTP in the CA1 field and learning and memory (15, 16). These findings suggest that the amygdala can influence stress-induced hippocampal function through inhibitory regulatory mechanisms.

Recently, Lee et al. (17) reported that amygdala-related,

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fear-associated learning was altered by prenatal stress exposure: Maternally stressed animals exhibited low levels of freezing behavior during the CFC retention session, a deficit in the maintenance of LTP at amygdala synapses, and decreases in glucocorticoid receptor expression in the amygdala. Based on these findings, the authors suggested that long-term deficits in amygdala function impair fear memory consolidation (17). Interestingly, we also observed that early postnatally stressed animals that received aversive footshock (FS) stimuli during postnatal days 14 to 18 (2nd week FS [2wFS] group) exhibited a marked reduction in freezing behavior during the CFC retention session (4, 18). Furthermore, decreases in synaptic transmission in the CA1 field during the CFC retention session did not occur in the 2wFS group (4). These findings led us to hypothesize that aversive stress exposure during the second postnatal period causes alterations in synaptic function in the CA1 field associated with fear memory, with the involvement of the amygdala. Based on this assumption, we investigated the possible involvement of the amygdala in hippocampal synaptic function, including metaplasticity, in the 2wFS group. Additionally, molecular biological approaches were used to evaluate extracellular signal–regulated kinase (ERK) signaling, considered important for synaptic plasticity and learning processes, including fear conditioning (19, 20).

Materials and Methods

Animals

Adult male Wistar rats (11 – 14-week-old) were used. Rats were housed in a room with a 12-h light/dark cycle with constant temperature (22°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.

Early postnatal stress

Rat pups were divided into two groups: a non-stressed control group (control) and a juvenile stress model group (21). Briefly, the pups received an aversive FS (shock intensity, 1.0 mA; intershock interval, 28 s; shock duration, 2 s) for 5 days using a FS box (Freeze Frame-41; Neuroscience Co., Ltd., Tokyo) combined with an automated analysis system (LimeLight-1, Neuroscience Co.) during the second postnatal week (PND 14 – 18, 2wFS group). We chose at least two pups from each litter to serve as controls. Non-FS controls remained in the FS box for the same time period (12.5 min) without FS stimuli for 5 days. Rats were housed 2 – 4 pups per cage after weaning (PND 28). During the postadolescent period (11 – 14 weeks), electrophysiological approaches, behavioral analysis, and immunobiochemical studies were performed based on CFC paradigms.

CFC test

At postadolescent period, rats were subjected to CFC, that is, rats were acclimated to the FS box for 5 min, subjected to the five FS (shock intensity, 1 mA; intershock interval, 28 s; shock duration, 2 s), and remained further for 5 min without FS stimuli. Twenty four hours later, the rats were re-exposed to the conditioning chamber (FS box) without FS stimuli. The 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, specially constructed by Nihon Kohden Corp., (Tokyo) for a simultaneous determination of electrophysiological and behavioral parameters. Freezing behavior was determined immediately after FS conditioning for 5 min (acquisition session) and during exposure to the FS box for 15 or 30 min (retention session). Presence or absence of freezing was determined each 5 s.

Electrophysiological experiments

Electrophysiological experiments under freely moving condition: Under pentobarbital (60 mg/kg, i.p.) anesthesia, a recording electrode was stereotaxically lowered into the CA1 field (5.0-mm posterior, 3.0-mm lateral to the bregma, approximately 2.3-mm ventral to the dura), and a bipolar stimulating electrode with a tip separation of 500 μm was placed in the Schaffer collaterals (3.0-mm posterior, 1.5-mm lateral to the bregma, 2.8-mm ventral to the dura) via holes drilled on the skull according to the atlas of Paxinos and Watson (22). After confirmation of the appropriate evoked potential, the stimulating and recording electrodes were implanted in the right ipsilateral side. The potential evoked by test stimulation (pulse duration, 250 μs; stimulus interval, 30 s) was monitored with an oscilloscope (VC-10, Nihon Kohden). The evoked potential in the CA1 field disappeared if the stimulating electrode was positioned outside of the appropriate stimulating region. The electrodes were anchored by small, electrically grounded screws and affixed to the skull with quick, self-curing acrylic resin (UNIFAST; GC Corp., Tokyo). Five days later, electrophysiological experiments combined with behavioral analysis were performed under freely moving conditions: The cable from the recording electrode was connected to an amplifier (MEG-5200, Nihon Kohden). Stimulating electrodes were connected through a cable to an electric stimulator (SEN-3301, Nihon Kohden) and an isolator (SS-202J, Nihon Kohden). The population spike ampli-

tude (PSA) was obtained from five successive stimuli and was recorded every 5 min with the PowerLab Data analysis system (AD Instruments, Pty., Ltd., Bella Vista, NSW, Australia). The intensity of the test stimulation was adjusted for each rat to elicit PSA of approximately 50% maximum amplitudes as assessed by input–output curves. High frequency stimulation [i.e., tetanus; 10 trains; each train consisted of 10 pulses; pulse duration (pulse width), 250 μs; interpulse interval, 10 ms; intertrain interval, 10 s] was applied after CFC retention. 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 electrode placement.

Electrophysiological experiments under anesthesia: To evaluate the homosynaptic metaplasticity, a recording electrode was lowered into the CA1 field and a bipolar stimulating electrode was placed in the Schaffer collaterals according to the method described above under urethane anesthesia (1 g/kg, i.p.). PSA in the CA1 field stimulated by the Schaffer collaterals (pulse duration, 250 μs) was obtained from five successive stimuli (stimulus interval, 30 s) and was recorded every 5 min with the PowerLab Data analysis system (AD Instruments). LFS (1 Hz for 15 min) was given prior to LTP-inducing tetanic stimulation (10 trains; each train consisted of 10 pulses; pulse duration, 250 μs; interpulse interval, 10 ms; intertrain interval, 10 s) in some rats. LFS and tetanus were given at the same intensity as the test stimulus.

The synaptic characteristics in the amygdala was evaluated by the long-term potentiation (LTP) induction in the amygdala – medial prefrontal cortex (mPFC) pathway and hippocampal (subicular region) – mPFC pathway. LTP in the mPFC (3.3-mm anterior, 0.8-mm lateral to the bregma, approximately 3.3-mm ventral to the dura) was induced by tetanic stimulation (3 sets of 10 trains; each train consisted of 10 pulses; pulse duration, 250 μs; interpulse interval, 10 ms; intertrain interval, 200 ms; interset interval, 1 min) of the basolateral amygdala (BLA; 3.3-mm posterior, 5.4-mm lateral to the bregma, approximately 7.0 – 9.0 mm ventral to the dura) (23). In the hippocampal–mPFC pathway, LTP in the mPFC (3.3-mm anterior, 0.8-mm lateral to the bregma, approximately 3.3-mm ventral to the dura) was induced by tetanic stimulation (2 sets of 10 trains; each train consisted of 50 pulses; pulse duration, 250 μs; interpulse interval, 4 ms; intertrain interval, 10 s) of the hippocampal CA1 subicular region (6.0-mm posterior, 5.6-mm lateral to the bregma, approximately 4.0 – 5.5-mm ventral to the dura) (24).

Amygdala modulation

To evaluate the possible involvement of the amygdala modulation on hippocampal synaptic plasticity, the amygdala was pharmacologically inactivated and electrically activated, in the control and the postnatally stressed rats, respectively. Pharmacological inactivation of the amygdala was accomplished by infusing the γ-aminobutyric acid-A ($GABA_A$)-receptor agonist muscimol (0.3 μmol; Sigma-Aldrich, Tokyo) into the BLA (3.3-mm posterior, 5.4-mm lateral to the bregma, approximately 8-mm ventral to the dura) using a modified dialysis probe through a guide cannula at a flow rate of 0.1 μl/min over a 3-min period. Muscimol was infused 20 min prior to the LFS or CFC retention session. Electrical stimulation of the BLA (3 sets of 10 trains; each train consisted of 10 pulses; intensity, 300 μA; pulse duration, 250 μs; interpulse interval, 10 ms; intertrain interval, 200 ms; interset interval, 1 min) was performed 1 min prior to the CFC retention session. After the experiments, the lesion site or location of the stimulation were confirmed histologically.

Immunoblotting experiment

The brain was dissected, rapidly cooled on ice, and sliced to a 500-μm thickness using a brain slicer (Linearslicer Pro 7; Dosaka EM, Kyoto). The BLA was taken from the sliced sections using a Pasteur pipette (1-mm inner diameter). The entire cell lysate of each BLA area was prepared using cell lysis buffer (Cell Signaling Technology, Beverly, MA, USA). The brain sample was soaked in cell lysis buffer, sonicated on ice, and centrifuged at 12,000 × g at 4°C for 10 min. The supernatant was used as the whole cell lysate. An 8-μg sample was separated on a 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). The membrane was probed with the primary antibodies and developed with horseradish peroxidase–conjugated secondary antibody by enhanced chemiluminescence. Anti-phospho-ERK1/2 (Thr^{202}/Tyr^{204}) antibody, anti-ERK1/2 antibody, anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibody (14C10), and peroxidase-conjugated anti-rabbit IgG antibody were purchased from Cell Signaling Technology. The luminescence intensity of specific bands was quantified using the luminescent image analyzer AE-6960 Light-Capture and CS Analyzer version 3.00 software (ATTO Corporation, Tokyo). The intensity relative to the mean intensity of each immunoblotting is shown as relative intensity.

Statistics

Data are expressed as the mean ± standard error of mean (S.E.M.). Electrophysiology data are expressed as the percentage of baseline values before LFS, CFC, or tetanus. The area under the curve (AUC: %·min × 10^3) of

the time-course changes was determined to evaluate the ensemble effects of PSA for 0 – 15 min and 15 – 60 min. Freezing behavior was expressed as the percentage of counts determined in 15- or 30-min blocks. The unpaired Student's *t*-test was used to compare data in the non-FS controls (control) with the 2wFS group or untreated groups with those treated groups. Values of $P < 0.05$ were considered statistically significant.

Results

Hippocampal synaptic plasticity associated with fear conditioning

We first examined fear-related freezing behavior and the synaptic response in the CA1 field induced by CFC under freely moving conditions. Consistent with our previous reports (4, 18), significant decreases in the expression of freezing were observed during the CFC retention session in the 2wFS group (mean ± S.E.M., 29.3% ± 4.9%, n = 6) compared with non-FS controls (61.4% ± 4.9%, n = 6, $P < 0.05$). High-frequency stimulation (tetanus)-induced LTP was completely suppressed after the CFC retention session in non-FS controls. The suppression of LTP did not occur in the 2wFS group; instead, synaptic enhancement was observed (Fig. 1).

Changes in metaplasticity in the CA1 field in the 2wFS group

Given the similarities between the CFC-induced suppression of LTP and homosynaptic metaplasticity (10, 11), we next assessed the synaptic response caused by LFS prior to tetanus under anesthesia. Consistent with previous results under freely moving conditions (11), LTP was suppressed after LFS prior to tetanus in non-FS controls. In the 2wFS group, however, LTP was not impaired; instead, LTP was induced after LFS prior to tetanic stimulation (Fig. 2).

Involvement of the amygdala modulation in the metaplasticity in the CA1 field

The possible involvement of the amygdala in the mediation of hippocampal synaptic function was investigated, with a focus on metaplasticity in non-FS controls. As shown in Fig. 3, LTP suppression caused by LFS prior to tetanus was completely inhibited by reversible inactivation of the BLA induced by the $GABA_A$-receptor agonist muscimol. Thus, metaplastic changes in the CA1 field appeared to be under the modulatory influence of the amygdala. The synaptic changes observed in amygdala-inactivated rats were mimicked by the effects of LFS on subsequent LTP in the 2wFS group (Fig. 2).

Involvement of amygdala regulation in the fear-related behavior

Based on the electrophysiological findings, we examined whether the amygdala modulates fear-related behavior during the CFC retention session. Inactivation of the BLA induced by muscimol pretreatment dramatically reduced freezing behavior in non-FS controls, similar to the 2wFS group (Fig. 4A). In contrast, electrical stimulation of the BLA in the 2wFS group significantly increased freezing behavior compared with the sham-treated group (Fig. 4B). This stimulus condition seems to be enough to activate the amygdala, because it was normally applied for inducing LTP in the amygdala–mPFC pathway (23).

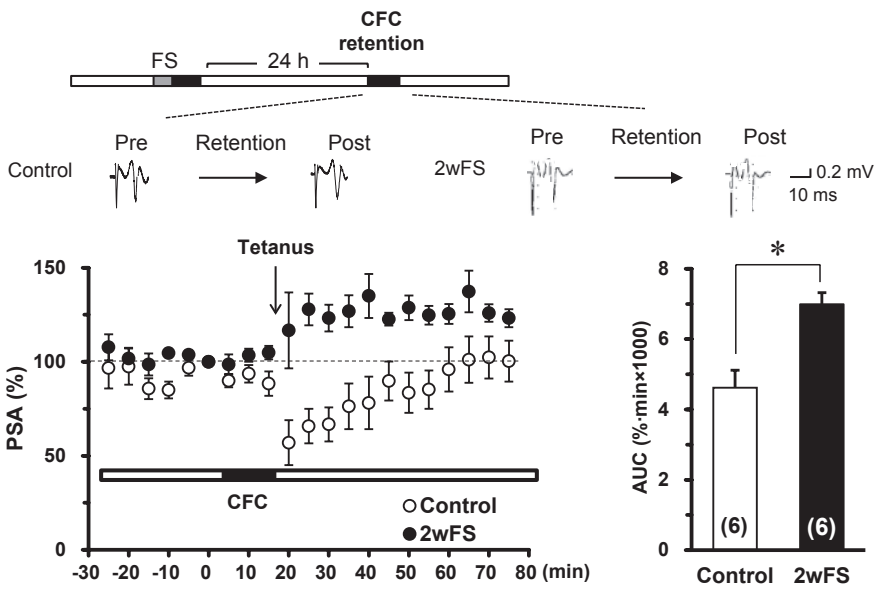

Fig. 1. Effects of early postnatal stress on synaptic plasticity in the CA1 field associated with fear conditioning. Specimen recordings and time-course response (left) of high-frequency stimulation (tetanus)-induced long-term potentiation in the hippocampal CA1 field after the contextual fear conditioning (CFC) retention session under freely moving conditions. The area under the curve (AUC: %·min × 10^3) of the time-course changes was determined to evaluate the ensemble effects of the population spike amplitude (PSA) for 60 min after tetanic stimulation (right). 2wFS, rats exposed to aversive footshock stress during the second postnatal period. The number of rats is shown in each column. Each value represents the mean ± S.E.M. *$P < 0.05$.

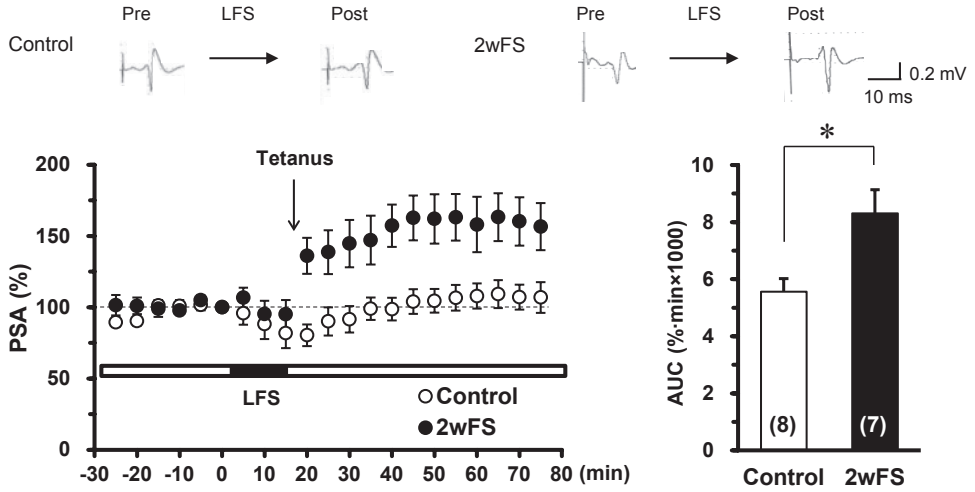

Fig. 2. Effects of early postnatal stress on metaplasticity in the CA1 field. Specimen recordings and time-course response (left) of the metaplasticity, that is, synaptic response induced by LFS (1 Hz for 15 min) prior to high-frequency stimulation (i.e., tetanus)-induced LTP in the CA1 field under anesthesia. The area under the curve (AUC: %·min × 10^3) of the time-course changes was determined to evaluate the ensemble effects of the population spike amplitude (PSA) for 60 min after tetanic stimulation (right). 2wFS, rats exposed to aversive footshock (FS) stress during the second postnatal period. The number of rats is shown in each column. Each value represents the mean ± S.E.M. *$P < 0.05$.

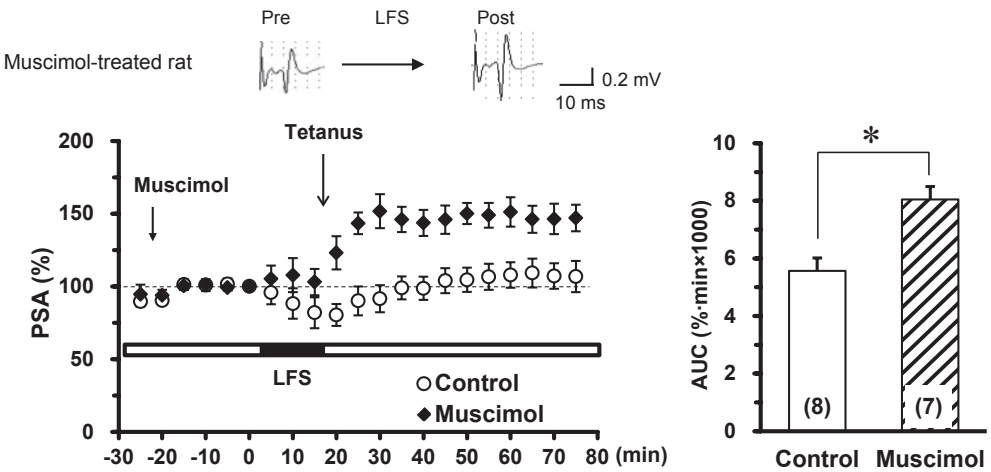

Fig. 3. Effects of amygdala inactivation on metaplasticity in the CA1 field. Specimen recordings and time-course response (left) of the metaplasticity, that is, synaptic response induced by low-frequency stimulation prior to tetanus-induced LTP in amygdala-inactivated rats. Inactivation of the amygdala was accomplished by microinfusing the $GABA_A$-receptor agonist muscimol (0.3 μmol) into the basolateral amygdala over a 3-min period. Twenty minutes later, LFS (1 Hz) was administered for 15 min, and then high-frequency stimulation (tetanus) was applied. The area under the curve (AUC: %·min × 10^3) of the time-course changes were determined to evaluate the ensemble effects of the population spike amplitude (PSA) for 60 min after tetanic stimulation (right). The number of rats is shown in each column. Each value represents the mean ± S.E.M. *$P < 0.05$.

ERK activity in the amygdala associated with fear conditioning

Molecular biological approaches were used to evaluate ERK signaling in the BLA associated with fear conditioning. In non-FS controls, phosphorylated ERK (pERK) levels in the BLA significantly increased after CFC retention. However, ERK activation was not found after the CFC retention session in the 2wFS group (Fig. 5). It seems likely that the pERK/ERK ratio in Ret (−) was higher in the 2wFS group. In addition, ERK activation in Ret (+) was significantly decreased compared to Ret (−) in the 2wFS group. These results led us to suppose that basal levels of ERK in the 2wFS group differ from those in the non-FS control. Nevertheless, the present data

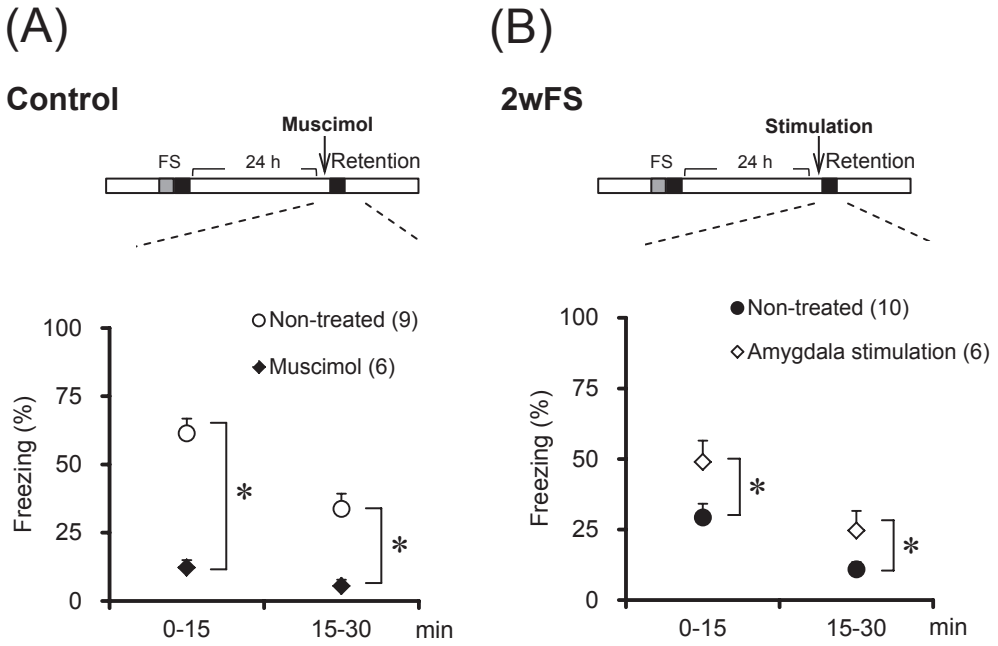

Fig. 4. Modulatory effects of the amygdala on freezing behavior during the contextual fear conditioning (CFC) retention session. Freezing behavior was determined during the CFC retention session after inactivation of the basolateral amygdala (BLA) in non-FS controls (A) or electrical stimulation of the BLA in the 2wFS group (B). The presence or absence of freezing behavior was estimated every 5 s for 30 min (retention). Inactivation of the amygdala was accomplished by infusing muscimol (0.3 μmol) into the BLA over a 3-min period. Twenty minutes later, the rats were re-exposed to the footshock (FS) box without FS stimuli (CFC retention session). Non-treated control, artificial cerebrospinal fluid was microinfused into the BLA. Electrical stimulation of the BLA (3 sets of 10 trains; each train consisted of 10 pulses; intensity, 300 μA; pulse duration, 250 μs; interpulse interval, 10 ms; intertrain interval, 200 ms; interset interval, 1 min) was performed 1 min prior to the CFC retention session. 2wFS, rats exposed to aversive footshock stress during the second postnatal period. Non-treated, sham-operated 2wFS group. The number of rats is shown in parentheses. Each value represents the mean ± S.E.M. *$P < 0.05$.

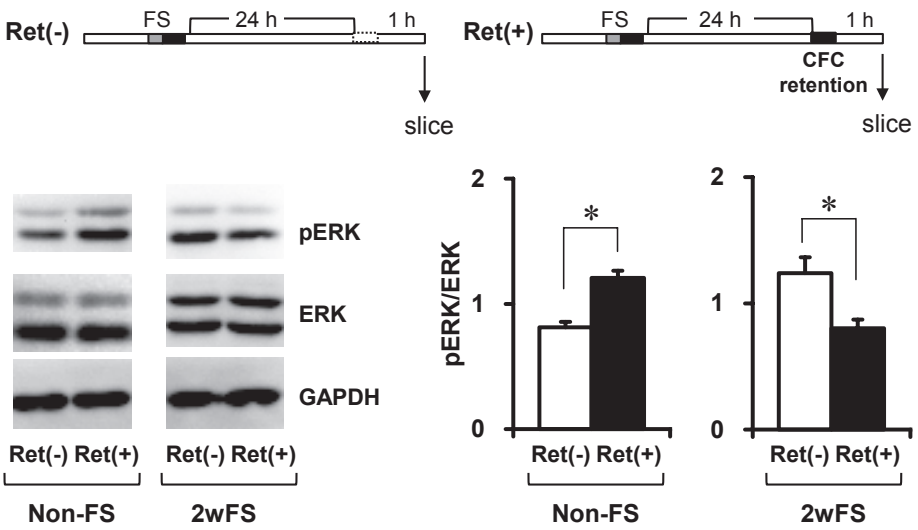

Fig. 5. ERK activity in the amygdala after contextual fear conditioning (CFC) retention session. Upper: Experimental protocol in the immunoblotting study. The basolateral amygdala (BLA) was dissected 1 h after CFC retention. Lower: Representative immunoblot and ERK activity in the BLA after CFC retention in non-FS control and the 2wFS group. ERK activation was assessed as the ratio of phosphorylation of ERK (pERK) to total ERK levels. GAPDH was used as an internal standard. 2wFS, rats exposed to aversive footshock (FS) stress during the second postnatal period. Ret, retention session of CFC. Each column represents the mean ± S.E.M. of four rats. *$P < 0.05$.

clearly showed downregulation of ERK signaling in the BLA associated with CFC in the 2wFS group.

Synaptic characteristics of the amygdala in the 2wFS group

To further investigate the characteristics of the

amygdala, LTP induction in the BLA–mPFC pathway was assessed by comparing it with the hippocampus (CA1 subicular region) – mPFC pathway. As shown in Fig. 6A, LTP in the BLA–mPFC pathway was induced in the 2wFS group, but its magnitude tended to time-dependently decrease. A significant difference was observed between groups in AUC of the PSA 15 – 60 min after tetanus (Fig. 6A). In contrast, no significant differences were found in the magnitude of LTP in the hippocampus–mPFC pathway between the 2wFS group and non-FS controls (Fig. 6B). These data suggest that impairment of LTP maintenance in the BLA–mPFC pathway in the 2wFS group is attributable to dysfunction of the amygdala.

Discussion

The present study demonstrated that early-life stress exposure during the second postnatal period (2wFS) impaired synaptic plasticity function, including metaplasticity, in the CA1 field. Consistent with previous studies (4, 18), fear-related freezing behavior was markedly reduced during CFC retention in the 2wFS group. The low levels of freezing unlikely resulted from deficits in the acquisition of fear conditioning because the expression of freezing immediately after FS conditioning was similar to non-FS controls. The low levels of freezing in the 2wFS group, therefore, are presumably attributable to disturbances in the consolidation or retrieval of

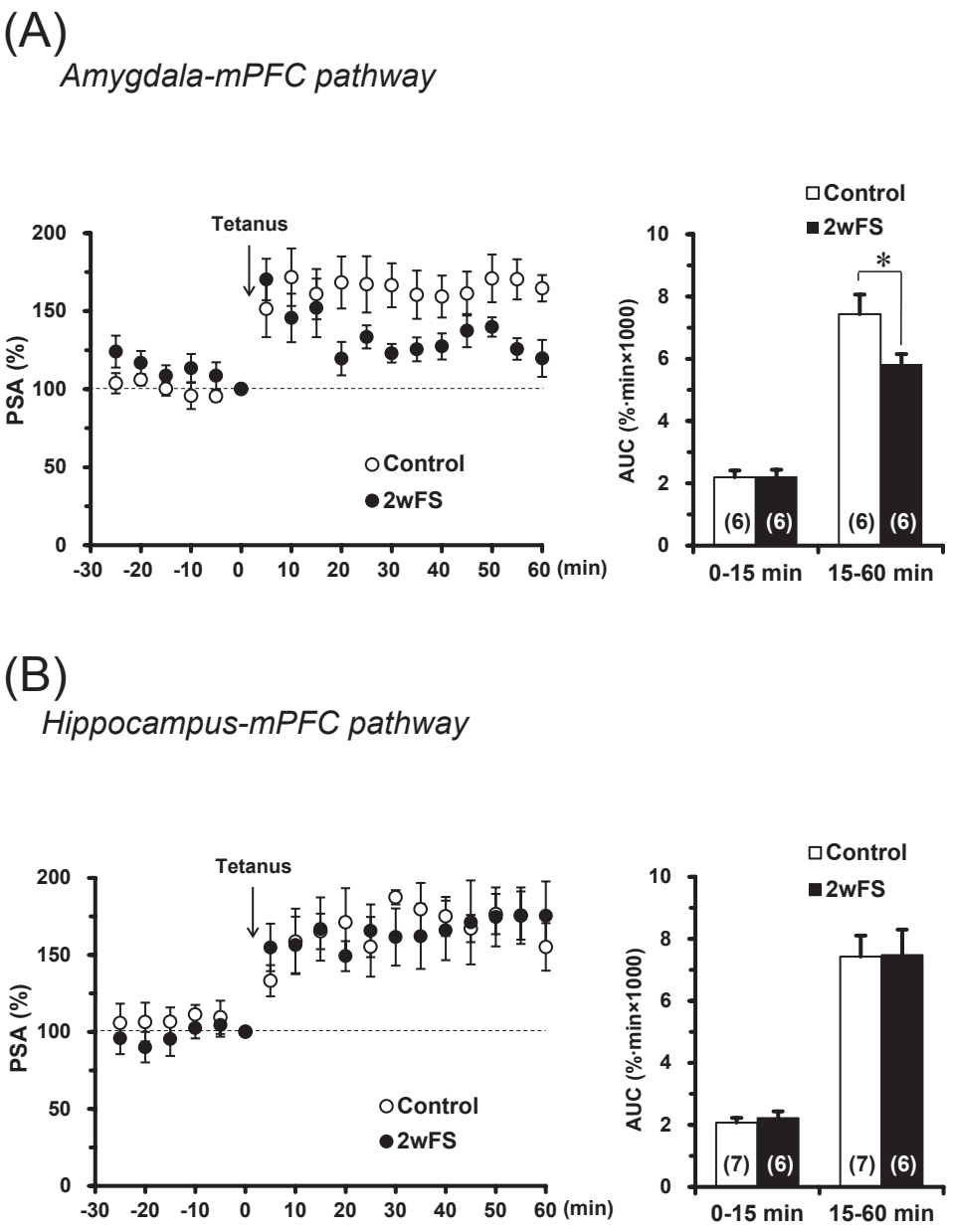

Fig. 6. Long-term potentiation in amygdala – medial prefrontal cortex (mPFC) pathway and hippocampus–mPFC pathway. A) Synaptic response in the amygdala–mPFC pathway was assessed by evoked potential in the mPFC by stimulation of the basolateral amygdala. Long-term potentiation was induced by high-frequency stimulation (tetanus). The time-course response (left) and area under the curve (AUC: %·min × 10^3) of the time-course changes were determined to evaluate the ensemble effects of the population spike amplitude for 0 – 15 and 15 – 60 min after tetanic stimulation (right). B) Synaptic response in the hippocampal–mPFC pathway was evaluated by evoked potential in the mPFC by stimulation of the CA1 subicular region. Long-term potentiation was induced by high-frequency stimulation (tetanus). The time-course response (left) and AUC of the time-course changes were determined to evaluate the ensemble effects of the PSA for 0 – 15 and 15 – 60 min after tetanic stimulation (right). 2wFS, rats exposed to aversive footshock (FS) stress during the second postnatal period. The number of rats is shown in each column. Each value represents the mean ± S.E.M. *$P < 0.05$.

fear memory. Similar observations were also reported in a recent study by Lee et al. (17): Maternally stressed mice exhibited low levels of freezing behavior during CFC retention. Based on electrophysiological and biochemical evidence, the authors suggested that prenatal stress–induced decreases in freezing behavior are attributable to the long-lasting impairment of fear memory consolidation but not acquisition that resulted from amygdala dysfunction (17).

The CFC-induced suppression of LTP that did not occur in the 2wFS group was mimicked by the results in which subsequent LFS-induced suppression of LTP was not observed; instead, LTP was induced in this group. Thus, the 2wFS group exhibited alterations in metaplasticity, in which prior synaptic activity influenced the subsequent induction of synaptic plasticity. To our knowledge, this is the first demonstration that metaplasticity in the CA1 field was impaired by early postnatal stress exposure. The behavioral changes that occurred during the CFC retention session in the 2wFS group, therefore, appear to be related to alterations in hippocampal synaptic function, including metaplasticity. These data support the hypothesis that metaplasticity in the CA1 field is a part of the neural basis of stress experience–dependent fear memory (8, 11).

In this study, metaplasticity was inhibited by muscimol-induced inactivation of the amygdala (BLA). Thus, the metaplastic changes in the CA1 field were heterosynaptically modulated by the amygdala. Although we cannot sufficiently explain the precise mechanism, metaplasticity in the hippocampal CA1 field may be directly or indirectly (via the entorhinal cortex) mediated by projections from the amygdala (25, 26). Hypothetically, activation of the amygdala triggers neuromodulatory systems that in turn may alter the threshold for LFS prior to tetanus. Interestingly, the alteration in metaplasticity induced by amygdala inactivation resembled the metaplasticity deficit observed in the 2wFS group. In other words, early postnatal stress exposure may affect the ability of amygdala priming to induce LFS-induced LTP in the CA1 field. Considering previous evidence of the contribution of the amygdala to the effects of stress on the hippocampal synaptic response (15, 16), the metaplasticity deficit in the 2wFS group may have resulted from amygdala dysfunction. This notion was partially supported by the data showing that maintenance of LTP in the amygdala–mPFC pathway was impaired in the 2wFS group. Furthermore, inactivation of the amygdala attenuated freezing behavior during CFC retention in the non-FS control, similar to the 2wFS group. Based on these findings, we speculate that early stress exposure during periods of amygdala development (27) might permanently influence the growth and maturation of amygdala–hippocampal neural circuits. This hypothesis was supported by the molecular biological data, in which ERK activation in the BLA in response to CFC did not occur in the 2wFS group. Altogether, early postnatal stress appears to cause dysfunction in the ability of the amygdala to mediate hippocampal synaptic functions that underlie contextual encoding, thereby resulting in disturbances in fear memory consolidation.

However, we cannot completely exclude the possible contribution of the mPFC in the functional linkage between the amygdala and hippocampus. The mPFC directly projects to the central nucleus of the amygdala, which in turn projects to the hippocampus (28) and also sends excitatory projections to the hippocampus via the entorhinal cortex, an area that is also involved in context-specific fear memory (29). Indeed, electrical stimulation of the mPFC decreased fear-related behavior (30) and counteracted the suppression of LTP induced by LFS prior to tetanus (11). However, the mPFC unlikely contributed to the synaptic changes and behavioral alterations observed in the 2wFS group because the naive characteristics of the mPFC in the 2wFS group did not differ from non-FS controls: The magnitude of LTP and input–output curves in the hippocampus–mPFC pathway were similar between these two groups. Additionally, ERK activity in the mPFC was not altered after the CFC retention session in either non-FS controls or the 2wFS group (31). These findings suggest that the mPFC is not involved in the functional interactions between the hippocampal CA1 field and amygdala, at least under the present experimental conditions.

In summary, the present study suggests that early postnatal stress causes long-lasting changes in the modulatory influence of the amygdala on hippocampal synaptic function that influence sensitivity to emotional stress. It has been suggested that early life stress experience alters the sensitivity to stress exposure later in life and contributes to the development of stress-related disorders such as depression and anxiety (32, 33). Although we do not yet know the precise association between low anxiety-like behavior (i.e., decreased freezing) observed in the 2wFS group and specific psychiatric disorders, the present data indicate that the second postnatal week is clearly a critical time for establishing lifelong "proper" emotional expression. Elucidating the regulatory effect of the amygdala on hippocampal function in early postnatally stressed rats, therefore, may provide insights into the functional role and pathological basis of the neural circuits that underlie stress-related mnemonic symptoms.

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References

1 Eldridge LL, Knowlton BJ, Furmanski CS, Bookheimer SY, Engel SA. Remembering episodes: a selective role for the hippocampus during retrieval. Nat Neurosci. 2000;3:1149–1152.
2 Corcoran KA, Maren S. Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. J Neurosci. 2001;21:1720–1726.
3 Tada K, Kasamo K, Suzuki T, Matsuzaki Y, Kojima T. Endogenous 5-HT inhibits firing activity of hippocampal CA1 pyramidal neurons during conditioned fear stress-induced freezing behavior through stimulating 5-HT_{1A} receptors. Hippocampus. 2004;14: 143–147.
4 Koseki H, Matsumoto M, Togashi H, Yamaguchi T, Izumi T, Yoshioka M, et al. Effects of aversive stress during brain development on hippocampal synaptic and behavioral responses to emotional stress at postadolescence. Jpn J Neuropsychopharmacol. 2007;27:19–27.
5 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. Psychopharmacol (Berl). 2005;179:606–612.
6 Matsumoto M, Togashi H, Ohashi S, Tachibana K, Yamaguchi T, Yoshioka M. Serotonergic modulation of psychological stress-induced alternation in synaptic plasticity in the rat hippocampal CA1 field. Brain Res. 2004;1022:221–225.
7 Abraham WC, Bear MF. Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci. 1996;19:126–130.
8 Abraham WC. Metaplasticity: tuning synapses and networks for plasticity. Nat Rev Neurosci. 2008;9:387–399.
9 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.
10 MacDonald JF, Jackson MF, Beazely MA. G protein-coupled receptors control NMDARs and metaplasticity in the hippocampus. Biochim Biophys Acta. 2007;1768:941–951.
11 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.
12 Le Doux JE. Emotion circuits in the brain. Annu Rev Neurosci. 2000;23:155–184.
13 Anglada-Figueroa D, Quirk GJ. Lesions of the basal amygdala block expression of conditioned fear but not extinction. J Neurosci. 2005;25:9680–9685.
14 Vouimba RM, Richter-Levin G. Physiological dissociation in hippocampal subregions in response to amygdala stimulation. Cereb Cortex. 2005;15:1815–1821.
15 Kim JJ, Koo JW, Lee HJ, Han JS. Amygdalar inactivation blocks stress-induced impairments in hippocampal long-term potentiation and spatial memory. J Neurosci. 2005;25:1532–1539.
16 Richter-Levin G. The amygdala, the hippocampus, and emotional modulation of memory. The Neuroscientist. 2004;10:31–39.
17 Lee EJ, Son GH, Chung S, Lee S, Kim J, Choi S, et al. Impairment of fear memory consolidation in maternally stressed male mouse offspring: Evidence for nongenomic glucocorticoid action on the amygdala. J Neurosci. 2011;31:7131–7140.
18 Matsumoto M, Higuchi K, Togashi H, Koseki H, Yamaguchi T, Kanno M, et al. Early postnatal stress alters the 5-HTergic modulation to emotional stress at postadolescent periods of rats. Hippocampus. 2005;15:775–781.
19 Atkins CM, Selcher JC, Petraitis JJ, Trzaskos JM, Sweatt JD. The MAPK cascade is required for mammalian associative learning. Nat Neurosci. 1998;1:602–609.
20 Guan Z, Peng X, Fang J. Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. Brain Res. 2004;1018:38–47.
21 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.
22 Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd ed. New York: Academic Press; 1986.
23 Maroun M, Richter-Levin G. Exposure to acute stress blocks the induction of long-term potentiation of the amygdala-prefrontal cortex pathway in vivo. J Neurosci. 2003;23:4406–4409.
24 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.
25 Krettek JE, Price JL. Projections from the amygdaloid complex and adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. J Comp Neurol. 1977;172: 723–752.
26 Pikkarainen M, Rönkkö S, Savander V, Insausti R, Pitkänen 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.
27 Bouwmeester H, Wolterink G, van Ree JM. Neonatal development of projections from the basolateral amygdala to prefrontal, striatal, and thalamic structures in the rat. J Comp Neurol. 2002;442:239–249.
28 McDonald AJ, Mascagni F, Guo L. Projections of the medial and lateral prefrontal cortices to the amygdala: a *Phaseolus vulgaris* leucoagglutinin study in the rat. Neurosci. 1996;71:55–75.
29 Hebert AE, Dash PK. Plasticity in the entorhinal cortex suppresses memory for contextual fear. J Neurosci. 2004;24: 10111–10116.
30 Milad MR, Vidal-Gonzalez I, Quirk GJ. Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. Behav Neurosci. 2004;118:389–394.
31 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 rats. Eur J Neurosci. 2012;35:135–145.
32 Scheller-Gilkey G, Moynes K, Cooper I, Kant C, Miller AH. Early life stress and PTSD symptoms in patients with comorbid schizophrenia and substance abuse. Schizophr Res. 2004;69: 167–174.
33 Myers KM, Davis M. Mechanisms of fear extinction. Mol Psychiatry. 2007;12:120–150.