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# Changes in amygdala neural activity that occur with the extinction of context-dependent conditioned fear stress

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ABSTRACT

The purpose of the present study was to characterize functional changes in the amygdala that accompany the extinction of context-dependent conditioned fear stress in a rat, an animal model of anxiety. Specifically, the effect of extinction of conditioned fear-induced cyclic AMP responsive element-binding protein (CREB) phosphorylation in the amygdala was investigated using immunohistochemistry. Experiments demonstrated that CREB phosphorylation in the basal nucleus of the amygdala decreased with the extinction of context-dependent conditioned fear-induced freezing behavior. These data suggest that the basal nucleus of the amygdala plays an essential role in the expression of context-dependent conditioned fear. Further, this is the first study to demonstrate that CREB phosphorylation in the basal nucleus of the amygdala changes in parallel with the extinction of context-dependent conditioned fear.



## 1. Introduction

Past studies have demonstrated that the amygdala plays a crucial role in anxiety and fear (Ono and Nishijo, 1992; LeDoux, 2000) and that the amygdala may be a target for the action of various kinds of anxiolytic drugs (Beck and Fibiger, 1995; Menard and Treit, 1999; Inoue et al., 2004). We recently reported that conditioned fear stress (CFS), an animal model of anxiety in rats, specifically induced c-Fos expression in the basal nucleus of the amygdala and that the administration of citalopram, a selective reuptake inhibitor, attenuated this increase in c-Fos expression (Izumi et al., 2006).

Conditioned fear stress is a type of classical conditioning (Fanselow, 1980) distinguished by acquisition, expression, and extinction (Myers and Davis, 2002). Acquisition occurs when a sensory stimulus (CS, conditioned stimulus), such as light, tone, or exposure to the test box (context), is paired with an aversive stimulus (US, unconditioned stimulus), such as footshock. Expression occurs when the animal is re-exposed to the CS without the US, and it elicits a variety of autonomic, hormonal, and behavioral conditioned responses. Extinction occurs when the CS is repeatedly presented in the absence of the US, and it decreases the amplitude of conditioned responses.

Extinction is thought to be an active learning process (Myers and Davis, 2002). Several studies have attempted to characterize the effect of a prefrontal cortex lesion on extinction, but the results have varied (Gewirtz et al., 1997; Morgan and LeDoux, 1999; Quirk et al., 2000). Further, administration of a *N*-methyl-D-aspartate (NMDA) receptor glycine site agonist facilitated extinction, while administration of a NMDA receptor antagonist, benzodiazepine receptor agonist, benzodiazepine receptor inverse agonist, muscarinic receptor antagonist, or dopamine-1 receptor agonist inhibited extinction (reviewed by Myers and Davis, 2002; Davis and Myers 2002).

The goal of the present study was to characterize changes in the amygdala neural activity that occur with the extinction of context-dependent CFS, using cAMP responsive element-binding protein (CREB) phosphorylation as an index of cellular activity.

## 2. Methods

This study was approved by the Hokkaido University School of Medicine Animal Care and Use Committee, and all protocols complied with the Guide for the Care and Use of Laboratory Animals of the Hokkaido University School of Medicine.

### 2.1. Animals

Male Sprague–Dawley rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan), weighing 250–300 g, were used. Four rats were housed per cage (38×33×17 cm), in a 12-h light:12-h dark cycle and a temperature-controlled environment (22±1 °C) with free access to food and water. Experiments were initiated after a 14-day adaptation period.

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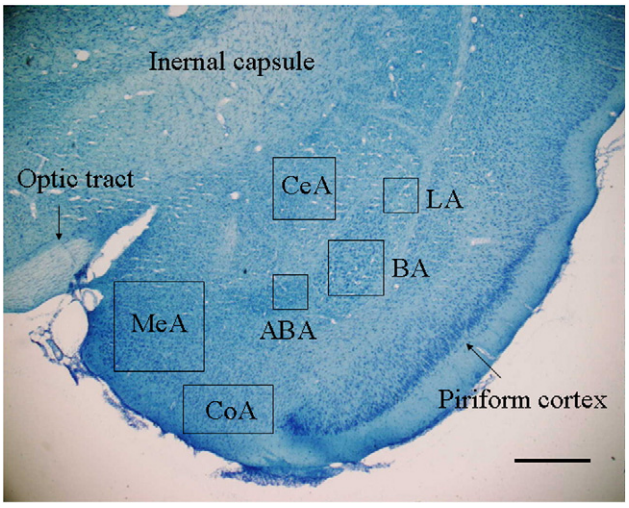


**Fig. 1.** Nissl staining of the amygdala (−3.14 mm to Bregma). BA, basal nucleus of the amygdala; ABA, accessory basal nucleus of the amygdala; CeA, central nucleus of the amygdala; CoA, cortical nucleus of the amygdala; LA, lateral nucleus of the amygdala; MeA, medial nucleus of the amygdala. Bar=500 μm.

### 2.2. CFS-induced freezing

Each rat was placed in a shock chamber (19×22×20 cm) and underwent 5 min of inescapable electric shocks (scrambled shocks of 0.2-mA intensity and 30-s duration, five times at variable intervals). Twenty-four hours after the footshock, the rats were again placed in the shock chamber and observed for 5 min without any shock application. During the 5-min observation period, freezing behavior was recorded using a time-sampling procedure (Fanselow, 1980), in which the animal behavior was classified as either "freezing" or "activity" at every 10-s interval. Freezing was defined as the lack of any observable movement of the body and the vibrissae, with the exception of movements related to respiration. Percentage scores for freezing were calculated for a 5-min observation period. Analysis of the freezing behavior was performed by an investigator who was blinded to the treatment.

### 2.3. Immunohistochemistry

Rats were anesthetized by pentobarbital injection (40 mg/kg, intraperitoneally) and perfused with saline and then by 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. The brains were sectioned at 30-μm thickness. Immunohistochemistry was performed on free-floating coronal sections (Umino et al., 1995). After 24-h incubation in 0.01 M phosphate-buffered saline and normal goat serum, the sections were incubated for 48 h in 0.01 M phosphate-buffered saline containing 0.2% Triton X-100 and rabbit anti-phospho-CREB antibody (Upstate Biotechnology, NY, 1:1000 dilution). The sections were incubated for 1 h in 0.01 M phosphate-buffered saline containing 0.2% Triton X-100 and biotinylated goat anti-rabbit IgG (Vector Labs) and then were incubated for 1 h in 0.01 M phosphate-buffered saline and avidin–biotinylated horseradish peroxidase complex (Vector Labs, Vectastain Elite ABC Kit). The reaction product was visualized by transferring the sections to a 50 mM Tris–HCl buffer (pH 7.6) containing 0.05% diaminobenzidine, 0.6% nickel ammonium sulfate and 0.01% $H_2O_2$.

### 2.4. Semiquantitative cell counting

According to the atlas of Paxinos and Watson (1997), the section that was located −3.14 mm posterior from the bregma was selected for semiquantitative evaluation of phospho-CREB (pCREB) immunoreactivity with a densitometric video image analysis system (MCID system, Imaging Research, CA, USA), according to the method of Bilang-Bleuel et al. (2002). The unit areas (200×200 μm) of the lateral nucleus, basal nucleus, accessory basal nucleus, central nucleus, medial nucleus, and cortical nucleus of the amygdala (Fig. 1) were digitally recorded by a CCD camera (CCD-IRIS, Sony, Japan) connected to a photomicroscope (B× 50, Olympus, Japan). The number of pCREB positive cells was assessed by automated selection of those cells within the unit areas that satisfied the following criteria: (1) the gray value of the cell nucleus was higher than the threshold value (threshold gray

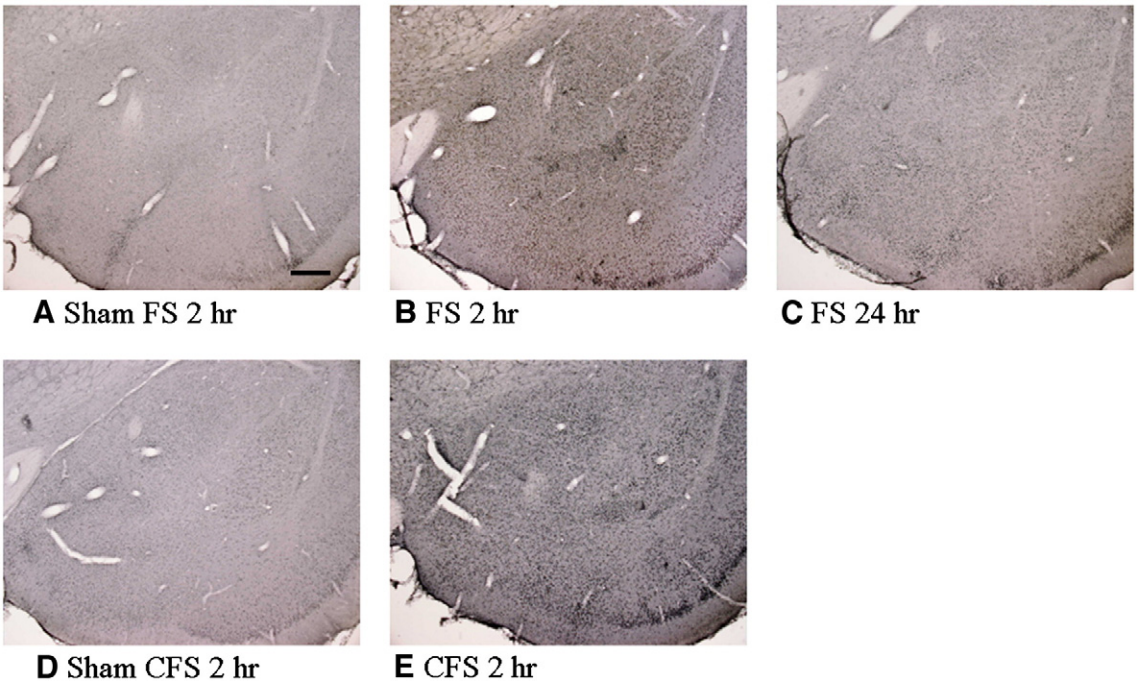


**Fig. 2.** Photomicrographs of the amygdala showing the expression of footshock and conditioned fear stress-induced phosphorylated CREB-like immunoreactivity. (A) 2 h after sham FS; (B) 2 h after FS; (C) 24 h after FS; (D) 2 h after sham CFS; (E) 2 h after CFS. FS, footshock; CFS, conditioned fear stress. Bar=200 μm.

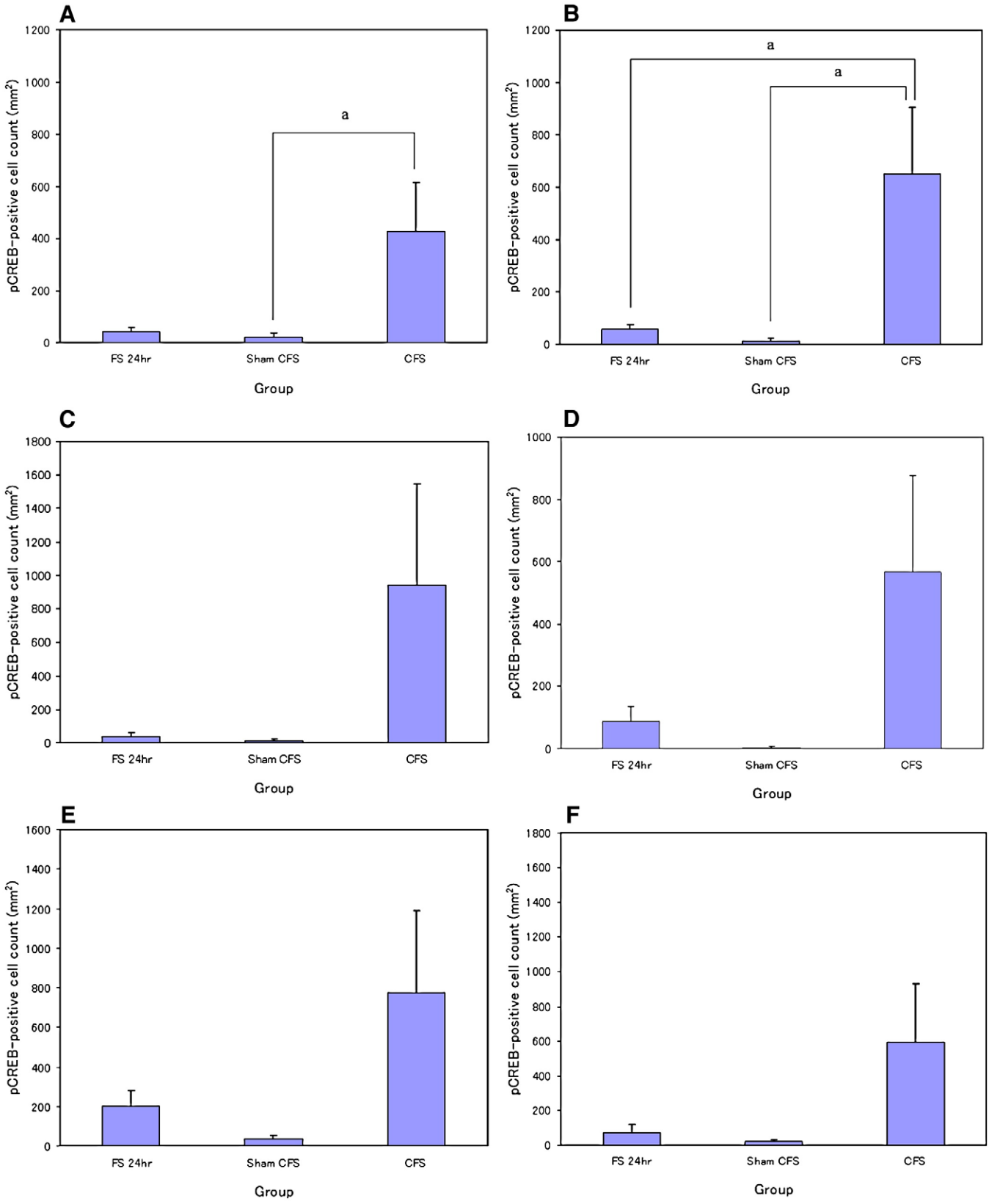


**Fig. 3.** Effect of sham footshock, footshock, sham conditioned fear stress, and conditioned fear stress on pCREB immunoreactivity in the amygdala of rats. Results are means with S.E.M. and are expressed as pCREB positive cell nuclei per mm$^2$. (A) lateral nucleus of the amygdala; (B) basal nucleus of the amygdala; (C) accessory basal nucleus of the amygdala; (D) central nucleus of the amygdala; (E) medial nucleus of the amygdala; (F) cortical nucleus of the amygdala. [a]$p<0.05$ The number of rats per group was as follows: FS 24 h group, $n=5$; sham CFS 2 h group, $n=6$; CFS 2 h group, $n=5$. FS, footshock; CFS, conditioned fear stress.

value=50% higher than the background gray value), (2) nuclei diameter of 4–12 μm (to exclude cell debris and artifacts). The background gray value was determined in a part of each unit area containing no nuclei. The measurement of the background gray value and the pCREB positive cell counting were repeated 3 times, and values were averaged. Semiquantitative cell counting was performed by an investigator who was blinded to the treatment.

### 2.5. Experimental procedures

#### 2.5.1. Experiment 1: CREB phosphorylation in the amygdala under various conditions

Amygdala CREB phosphorylation was assessed under various experimental conditions. The rats were assigned to one of the following 5 groups. (1) Sham footshock 2 h group: The rats were individually placed in the shock chamber without footshocks and 5 min later, they were returned to their homecages (sham footshock). One hour and 45 min later, they were anesthetized in their homecages and then perfused 15 min later: (2) Footshock 2 h group: After the 5-min footshock, the rats were returned to their homecages. One hour and 45 min later, they were anesthetized and perfused similarly: (3) Footshock 24 h group: After the 5-min footshock, the rats were returned to their homecages. Twenty-four hours later, they were anesthetized and perfused similarly: (4) Sham CFS 2 h group: The rats were individually placed in the shock chamber without footshocks and 5 min later, they were returned to their homecages. Twenty-four hours later, they were individually placed in the shock chamber again without footshocks and 5 min later, they were returned to their homecages (sham CFS). One hour and 45 min later, they were anesthetized and perfused similarly: (5) CFS 2 h group: After the 5-min footshock, the rats were returned to their homecages. Twenty-four hours later, they were individually placed in the shock chamber again without footshocks and 5 min later, they were returned to their homecages. One hour and 45 min later, they were anesthetized and perfused similarly. The number of rats per group was as follows: sham footshock 2 h, $n=6$; footshock 2 h, $n=2$; footshock 24 h, $n=5$; sham CFS 2 h, $n=6$; CFS 2 h, $n=5$. Because the number of rats in the footshock 2 h group was relatively small, statistical analysis was performed to compare only the footshock 24 h, sham CFS 2 h and CFS 2 h groups.

#### 2.5.2. Experiment 2: Effect of extinction on CFS-induced freezing behavior and CREB phosphorylation in the amygdala

Changes of CFS-induced freezing behavior and CREB phosphorylation in amygdala were assessed relative to the extinction process of CFS. Rats were assigned to one of the following 6 groups. (1) Sham CFS expression×1 group: One hour and 45 min after above-mentioned sham CFS, the rats were anesthetized and perfused similarly: (2) Sham CFS expression×2 group: Twenty-four hours after sham CFS, the rats were subjected to the second sham CFS. One hour and 45 min later, they were anesthetized and perfused similarly: (3) Sham CFS expression×3 group: Twenty-four hours after the second sham CFS, the rats were subjected to the third sham CFS. One hour and 45 min later, they were anesthetized and perfused similarly: (4) CFS expression×1 group: One hour and 45 min after CFS, the rats were anesthetized and perfused similarly: (5) CFS expression×2 group: Twenty-four hours after CFS, the rats were subjected to the second CFS. One hour and 45 min later, they were anesthetized and perfused similarly: (6) CFS expression×3 group: Twenty-four hours after the second CFS, the rats were subjected to the third CFS. One hour and 45 min later, they were anesthetized and perfused similarly. During the 5-min sham CFS or CFS operation, the behavior of the rats was recorded on videotape. In the behavior experiment, the number of rats per group was 6, and in the histological experiment, the number of rats per group was as follows: sham CFS expression×1–3 groups, $n=6$; CFS expression×1 group, $n=5$; CFS expression×2, 3 groups, $n=4$.

### 2.6. Data analysis

Multiple group comparisons were performed using one-way and two-way analysis of variance (ANOVA) and the Bonferroni–Dunn's post hoc test. Simple linear regression analysis was used to evaluate the correlations between percent freezing rate and pCREB positive cell count and between times of expression and pCREB positive cell count using the data from sham CFS expression×1–3 and CFS expression×1–3 groups.

## 3. Results

### 3.1. Experiment 1: CREB phosphorylation in the amygdala under various conditions

One-way ANOVA indicated a significant effect of CFS footshock compared to the footshock 24 h group and sham CFS group in the lateral nucleus ($F(2, 9)=4.9$, $p=0.026$) and basal nucleus ($F(2, 9)=6.6$, $p=0.011$), but not in the accessory basal nucleus, ($F(2, 9)=6.6$, $p=0.12$), central nucleus ($F(2, 9)=6.6$, $p=0.074$), medial nucleus ($F(2, 9)=6.6$, $p=0.093$) and cortical nucleus ($F(2, 9)=6.6$, $p=0.092$) of the amygdala (Fig. 2, Fig. 3A–3F). In the lateral nucleus, post hoc comparison showed that the number of pCREB positive cells was greater in the CFS 2 h group than in the sham footshock 2 h group ($p=0.014$) (Fig. 2, Fig. 3A). In the basal nucleus, post hoc comparison showed that the number of pCREB positive cells was greater in the CFS 2 h group than in the sham footshock 2 h group ($p=0.011$) and the footshock 24 h group ($p=0.0054$) (Fig. 2, Fig. 3B).

### 3.2. Experiment 2: Effect of extinction on CFS-induced freezing behavior

Two-way ANOVA (CFS×expression) indicated a significant main effect of CFS ($F(2, 66)=47.1$, $p<0.0001$) and expression ($F(2, 66)=19.0$, $p<0.0001$) in freezing behavior. Two-way ANOVA also indicated a significant interaction between CFS ($F(2, 66)=47.1$, $p<0.0001$) and expression ($F(2, 30)=21.1$, $p<0.0001$). One-way ANOVA across 6 groups indicated a significant effect of treatment ($F(5, 66)=36.3$, $p<0.0001$). Post hoc comparison indicated a significant difference between CFS expression×1 group and CFS expression×2 group ($p<0.0001$) and

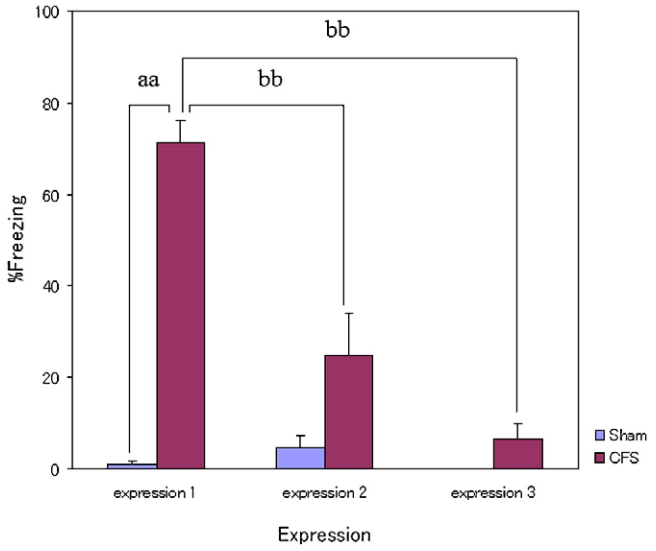


**Fig. 4.** Effect of repeated exposure to shock box on CFS-induced freezing behavior in rats. Results are the mean percentage with S.E.M. of freezing scored for a 5-min observation period. $^{aa}p<0.01$, $^{bb}p<0.01$, $n=6$. Expression 1: groups subjected to sham CFS or CFS one time. Expression 2: groups subjected to sham CFS or CFS two times. Expression 3: groups subjected to sham CFS or CFS three times. Sham, sham conditioned fear stress; CFS, conditioned fear stress; N.S., not significant.

between CFS expression×1 group and CFS expression×3 group ($p<0.0001$). Post hoc comparison also indicated significant difference between sham CFS expression×1 group and CFS expression×1 group ($p<0.0001$), but the difference between sham CFS expression×2 group and CFS expression×2 group and the difference between sham CFS expression×3 group and CFS expression×3 group were not statistically significant (Fig. 4).

### 3.3. Experiment 2: Effect of extinction on CFS-induced CREB phosphorylation in the amygdala

In the basal nucleus of the amygdala, two-way ANOVA (CFS×expression) indicated a significant main effect of CFS ($F(2, 25)=10.2$, $p=0.0037$) and a significant interaction between CFS and expression ($F(2, 25)=21.1$, $p<0.0001$), but the main effect of expression was not statistically significant ($F(2, 25)=10.2$, $p=0.10$). One-way ANOVA across 6 groups indicated a significant effect of treatment ($F(5, 25)=4.7$, $p=0.0037$) in the basal nucleus. Post hoc comparison indicated a significant difference between the sham CFS expression×1 group and CFS expression×1 group ($p=0.0003$), but the difference between the sham CFS expression×2 group and CFS expression×2 group and the difference between the sham CFS expression×3 group and CFS expression×3 group were not statistically significant (Fig. 5B). In the lateral and accessory basal nucleus of the amygdala, the main effects of CFS ($F(2, 25)=1.4$, $p=0.25$; $F(2, 25)=3.8$, $p=0.064$, respectively), the main effects of expression ($F(2, 25)=1.09$, $p=0.35$; $F(2, 25)=1.6$, $p=0.21$, respectively) and the interactions between CFS and expression ($F(2, 25)=2.8$, $p=0.080$; $F(2, 25)=1.8$, $p=0.19$, respectively) were not significant (Fig. 5A, C). In the central, medial and cortical nucleus of amygdala, two-way ANOVA (CFS×session) indicated that the main effects of CFS ($F(2, 25)=5.7$, $p=0.025$; $F(2, 25)=7.1$, $p=0.013$; $F(2, 25)=7.1$, $p=0.014$, respectively) were significant, but the main effects of expression ($F(2, 25)=1.8$, $p=0.19$; $F(2, 25)=1.6$, $p=0.22$; $F(2, 25)=1.4$, $p=0.28$, respectively) and the interactions between CFS and expression ($F(2, 25)=2.4$, $p=0.12$; $F(2, 25)=1.7$, $p=0.20$; $F(2, 25)=1.4$, $p=0.28$, respectively) were not significant. Post hoc comparisons of

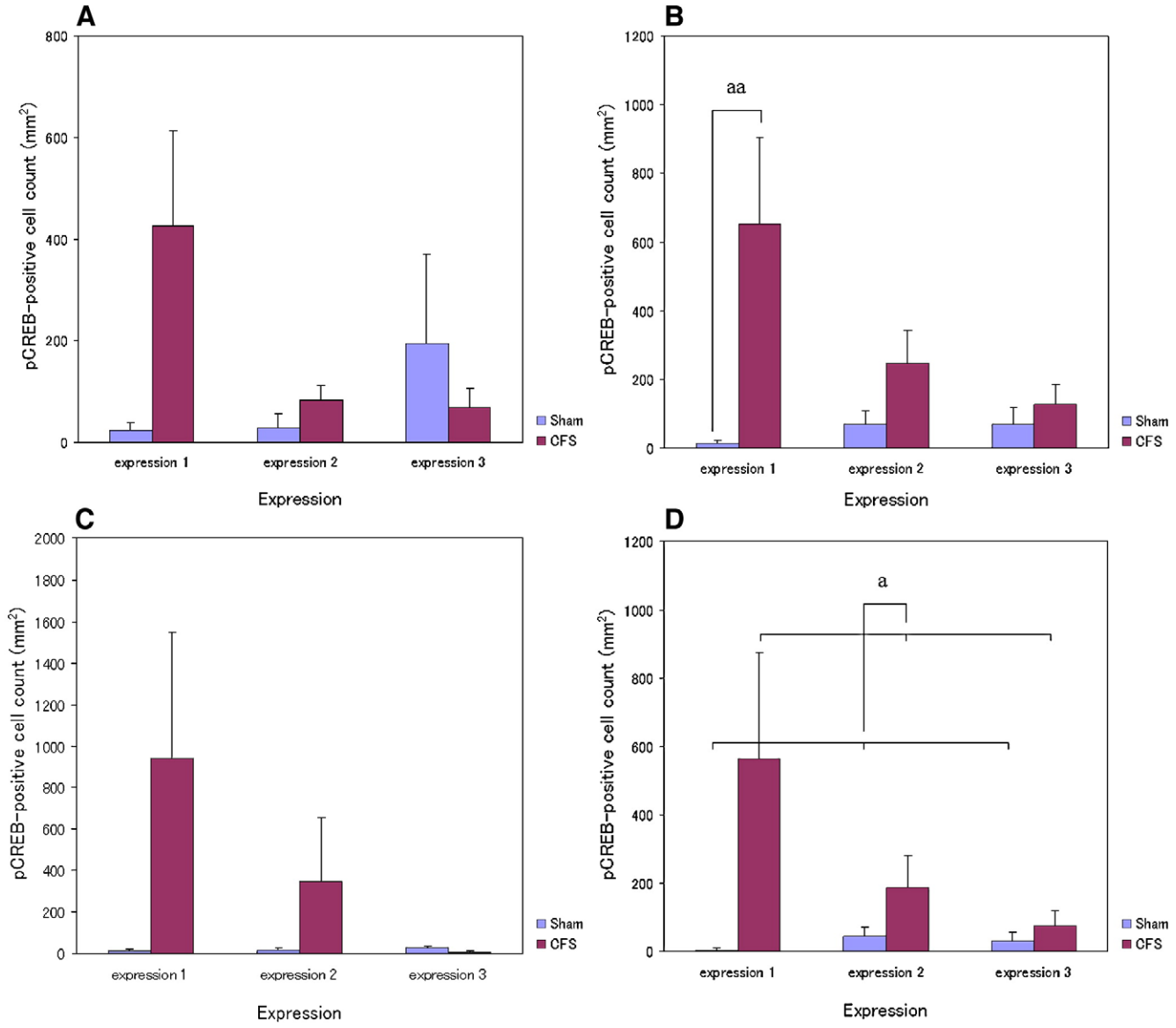


**Fig. 5.** Effect of repeated exposure to shock box on pCREB immunoreactivity in the amygdala of rats. Results are means with S.E.M. and are expressed as pCREB positive cell nuclei per $mm^2$. (A) lateral nucleus of the amygdala; (B) basal nucleus of the amygdala; (C) accessory basal nucleus of the amygdala; (D) central nucleus of the amygdala; (E) medial nucleus of the amygdala; (F) cortical nucleus of the amygdala. $^{a}p<0.05$, $^{aa}p<0.01$ The number of rats per group was as follows: sham CFS expression×1–3 groups, $n=6$; CFS expression×1 group, $n=5$; CFS expression×2, 3 groups, $n=4$. Expression 1: groups subjected to sham CFS or CFS one time. Expression 2: groups subjected to sham CFS or CFS two times. Expression 3: groups subjected to sham CFS or CFS three times. CFS, conditioned fear stress; Sham, sham conditioned fear stress; N.S., not significant.

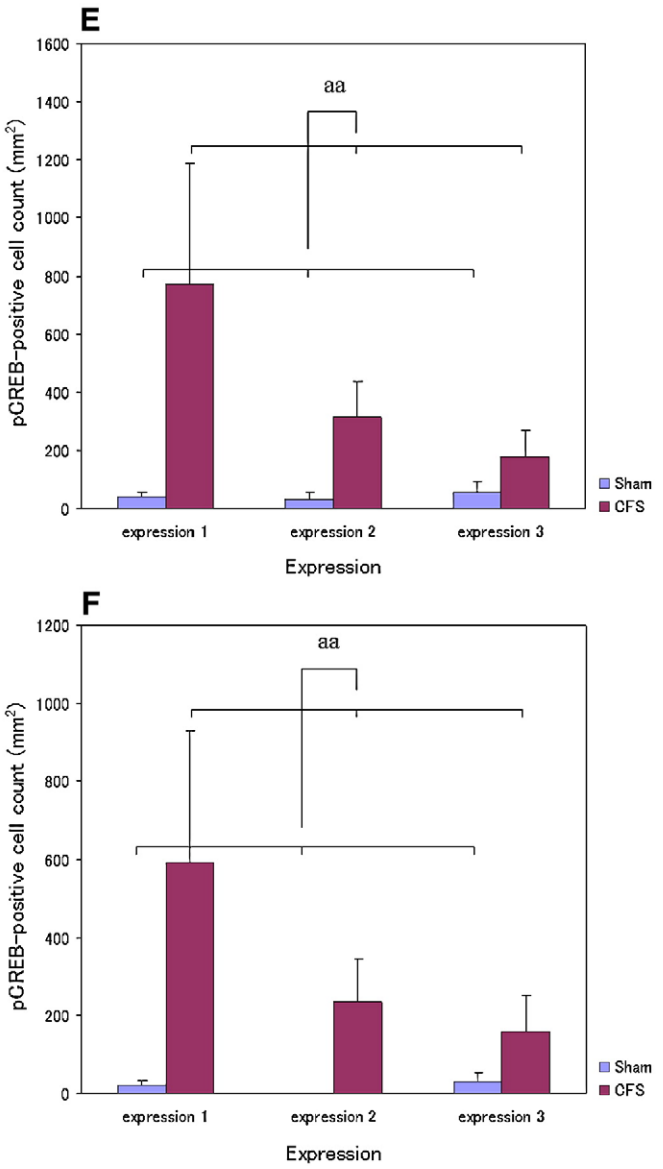


**Fig. 5** *(continued).*

two-way ANOVA indicated a significant difference between the sham CFS groups (expression×1 group+expression×2 group+expression×3 group) and CFS groups (expression×1 group+expression×2 group+expression×3 group) in these 3 nuclei ($p=0015$; $p=0.083$; $p=0.0088$, respectively) (Fig. 5D, E, F).

Regression analysis indicated a significant correlation between percent freezing rate and pCREB positive cell count in the lateral ($r=0.43$, $p=0.015$), basal ($r=0.63$, $p=0.001$), accessory basal ($r=0.51$, $p=0.031$), central($r=0.63$, $p=0.002$), medial ($r=0.55$, $p=0.0015$) and the cortical ($r=0.48$, $p=0.0064$) nucleus of amygdala, but there was no significant correlation between times of expression and pCREB positive cell count in any of the subnuclei of amygdala.

## 4. Discussion

Two different studies have investigated the time course of stress-induced CREB phosphorylation in brain. Stanciu et al. (2001) indicated that contextual-dependent conditioned fear resulted in two peaks of CREB phosphorylation in the parietal cortex, CA1, dentate, basolateral amygdala, and the central amygdala of mice. The first peak occurred 7 min after stress and disappeared at 90 min after stress. The second peak appeared at 180 min after stress and persisted at 360 min after stress. However, they did not investigate CREB phosphorylation at 120 min after stress. Bilang-Bleuel et al. (2002) also showed that forced swimming resulted in two peaks of CREB phosphorylation in

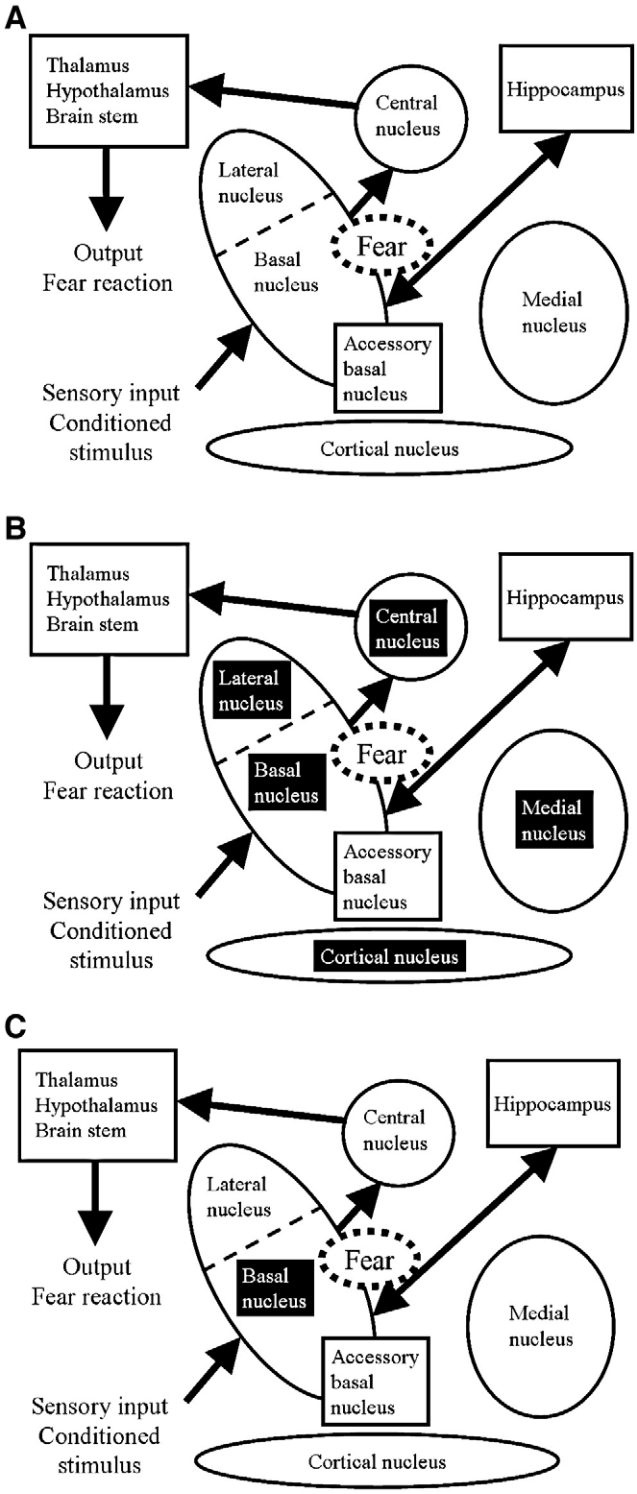


**Fig. 6.** Scheme representing the changes of CREB phosphorylation in amygdala subnuclei by the expression and extinction of contextual conditioned fear. (A) Neural pathway concerning the expression of contextual conditioned fear. (B) The region where CREB phosphorylation was increased by the expression of conditioned fear (indicated by white letters and black background). (C) The region where CREB phosphorylation was increased in association with the extinction of conditioned fear (indicated by white letters and black background).

the dentate and the neocortex in rats. The first peak occurred at 15 min after stress and disappeared at 60 min after stress. The second peak appeared at 120 min after stress and persisted at 48 h after stress. Based on these studies, the expectation was that stress-induced CREB phosphorylation would result in two peaks, the first within 1 h and the second at more than 2 h. However, the time point of peaks is variable according to the animal species, type of stress, intensity and duration of stress, and the brain location. Data from the present study reflect the second peak of CREB phosphorylation. Further study would be of benefit to investigate the difference in the biological mechanisms between these two peaks.

In Experiment 1, pCREB levels of the lateral and basal nucleus of the amygdala were also increased in the CFS 2 h group. Because amygdala pCREB levels did not increase in the footshock 24 h group, increases in pCREB levels in the amygdala in the CFS 2 h group were not considered to be the result of the footshock 24 h before. Moreover, amygdala pCREB levels in the sham CFS 2 h group did not increase, suggesting that placing rats in the shock chamber without administrating shocks itself did not play a role in this phenomenon. Thus, the increase in pCREB levels in the lateral and basal nucleus in CFS 2 h group likely reflect the neural process of the expression of context-dependent CFS. Indeed, Hall et al. (2001) reported expression of tone-dependent CFS-induced CREB phosphorylation in the lateral and basal nucleus of the amygdala in rats, which is consistent with data from the present study.

In Experiment 2, CFS-induced freezing behavior significantly decreased as the expression of CFS was repeated; this suggests that extinction of CFS occurred. CFS-induced CREB phosphorylation in the basal nucleus also decreased as the expression was repeated. While there was a significant difference between the sham CFS group and the CFS group in expression 1, there was no difference between these groups in expressions 2 and 3, which suggests that CREB phosphorylation in the basal nucleus decreased in parallel with the extinction of CFS-induced freezing behavior. In the central, medial and the cortical nucleus of amygdala, CREB phosphorylation was greater in CFS groups than in the sham CFS groups; however, no time-course effect was observed, as seen in the basal nucleus.

Experiments 1 and 2 demonstrated that CREB phosphorylation was increased by CFS in the lateral, basal, central, medial and the cortical nucleus of amygdala, but CREB phosphorylation was decreased in association with extinction of CFS only in the basal nucleus (Fig. 6B, C). Indeed, Lin et al. (2003) reported that the tone-dependent CFS-induced CREB phosphorylation in the amygdala including the lateral and basal nuclei, decreased as the extinction session was repeated in rats, which is at least partially consistent with our result.

In a previous study, we reported that CFS-induced c-Fos expression in the basal nucleus, but not in the central nucleus of amygdala (Izumi et al., 2006). Although other studies have reported that CFS-induced c-Fos expression in many cortical and subcortical region, the basal nucleus of amygdala was the common location that was associated with CFS (Izumi et al., 2006). Further, pCREB is upstream of c-Fos in the intracellular signal pathway, and pCREB enhances c-Fos production as well as production of other transcription factors. Therefore, functional mapping of pCREB is likely distinct from that of c-Fos. Regardless, the present and previous studies have consistently demonstrated a significant change in the basal nucleus with contextual CFS.

Anatomical and lesion studies have characterized the nuclei of the amygdala that participate in the expression of CFS. Anatomical study indicated that the lateral, basal and central nuclei of the amygdala receive moderate to heavy projections from the sensory-related cortices and the thalamus and that these nuclei are expected to mediate sensory input to the amygdala (Pitkanen, 2000). Further, the central and medial nuclei provide moderate to heavy projections to the subcortical nuclei and the brain stem, and these nuclei are expected to mediate motor, autonomic and hormonal output from the amygdala (Pitkanen, 2000).

Studies regarding tone-dependent CFS indicated that the tone stimulus is received at the auditory organ and is converted to neural information, which subsequently passes through the thalamus and auditory cortex. This neural information is received by the lateral nucleus, which subsequently induces a fear reaction via the central nucleus (LeDoux, 2000). In context-dependent CFS, the contextual stimulus (e.g., exposure to the shock chamber), is received at the sensory organ and is converted to the neural information, which subsequently passes through the thalamus and sensory-related cortices. After being checked against memory in the hippocampus, this information is received by the basal nucleus, which induces a fear reaction via the central nucleus (LeDoux, 2000) (Fig. 6A). In the present study, context-dependent CFS-induced CREB phosphorylation in the basal nucleus and in the lateral nucleus, suggesting that the sensory stimuli except context (e.g., tone or smell) is partly related to fear-conditioning.

Two different neural plasticity-related phenomena occur in brain after the fear memory is retrieved; reconsolidation and extinction. The effects of these two processes on rat behavior are contrary, the former maintains fear related behavior, while the latter reduces it when the fear memory retrieval is repeated (Nader et al., 2000; Debiec et al., 2002). In the present study, the percent freezing rate approximately 70% in the first retrieval, 20% in the second, and 10% in the third. The fact that freezing behavior reduced rapidly in parallel with levels of CREB phosphorylation suggests that the extinction process was dominant over the reconsolidation process in our experimental design. Thus, neural activity of the basal amygdala reflects the extinction process in the case of context-dependent aversive classical conditioning.

We previously reported that CFS-induced c-Fos expression in the basal nucleus (Izumi et al., 2006). The present study demonstrated that CREB phosphorylation in the basal nucleus of the amygdala decreased with the extinction of context-dependent conditioned fear-induced freezing behavior. These data suggest that the basal nucleus of the amygdala plays an essential role in the expression of context-dependent conditioned fear, and this is the first study to demonstrate that CREB phosphorylation in the basal nucleus of the amygdala changes in parallel with the extinction of context-dependent conditioned fear.

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