

Relationship between Fos Production and Classical Fear Conditioning: Effects of Novelty, Latent Inhibition, and Unconditioned Stimulus Preexposure

Jelena Radulovic, Jens Kammermeier, and Joachim Spiess

Max Planck Institute for Experimental Medicine, Department for Molecular Neuroendocrinology, 37075 Goettingen, Germany

The relationship between FOS production in the sensory cortex and limbic system and the ability of C57BL/6N mice to acquire context- and tone-dependent freezing were investigated after fear conditioning, which was achieved by exposure of mice to context only or context and tone (10 kHz, 75 dB) as conditioned stimuli (Cs) paired with an electric footshock (0.7 mA, constant) as unconditioned stimulus (Us). The effect of preexposure to Cs or Cs paired with Us on FOS production and learning was also tested. It was demonstrated that high simultaneous FOS production in the parietal cortex, hippocampus, and amygdala paralleled the ability of mice to acquire strong freezing responses to novel Cs. After contextual preexposure (latent inhibition), FOS production could be elicited in the central amygdala only by shock and in the basolateral amygdala only by tone. Under these conditions, the ability of mice to acquire

contextual freezing was almost abolished, whereas tone-dependent freezing was reduced. Lacking FOS production in the central amygdala after preexposure to context followed by shock (Us preexposure effect) paralleled the inability of mice to acquire tone-dependent freezing, although the tone elicited FOS production in the basolateral amygdala. On the basis of these findings it was concluded that synchronous Cs- and Us-induced FOS production in several defined forebrain areas was accompanied with associative learning of novel stimuli, and that a subsequent low level of FOS production might have been responsible or indicative for delayed conditioning to those stimuli.

Key words: fear conditioning; latent inhibition; Us preexposure; FOS; amygdala; hippocampus; parietal cortex; mice; C57BL/6N

Context- and tone-dependent fear conditioning may be rapidly induced in rodents after exposure to novel conditioned (Cs) and unconditioned (Us) stimuli (Blanchard and Blanchard, 1969; Bolles and Collier, 1976). However, fear conditioning may be markedly delayed by nonreinforced presentation of phasic and contextual stimuli when the same stimuli are subsequently presented with a Us, a phenomenon described as latent inhibition. Similarly, preexposure to a Us in a defined context markedly reduces its reinforcing strength when the same Us is later presented with a Cs (Baker and Mackintosh, 1979).

We have reported previously that C57BL/6N mice acquire a strong freezing response after context- and tone-dependent fear conditioning performed in a single trial (Stiedl and Spiess, 1997; Radulovic et al., 1998). This paradigm was demonstrated to be advantageous in studying the molecular changes in the brain during learning because molecular events can be monitored during a defined time course of memory formation, repeated exposures to Cs and Us are avoided, and the effects of Cs and Us can be dissociated. By using FOS protein as a marker of neuronal activity (Sagar et al., 1988), we have demonstrated that FOS production in several forebrain areas is linked to acquisition of

foreground contextual fear conditioning (Milanovic et al., 1998). Recent investigations of synaptogenesis in FOS-producing neurons (Kleim et al., 1996) and the use of FOS antisense oligonucleotides (Mileusnic et al., 1996; Swank et al., 1996) provided evidence that induction of c-fos may represent a necessary step toward the formation of long-term memory. However, in our experiments FOS production did not correlate with the fear response after the memory test because of a marked reduction of FOS production that was observed in conditioned and control animals. In agreement with this observation, Campeau et al. (1997) demonstrated that c-fos expression in the hippocampus and amygdala of fear-conditioned and control rats did not differ. These results were in agreement with the finding that c-fos was downregulated after repeated exposure to the same stimuli (Papa et al., 1993; Chen and Herbert, 1995; Hess et al., 1995), but contrasted with observations that FOS production is increased after reexposure to a Cs (Campeau et al., 1991; Pezzone et al., 1992; Smith et al., 1992; Beck and Fibiger, 1995).

To obtain additional information on the significance of FOS production for acquisition of conditioned fear, we have investigated the FOS production and behavior of mice after context- and tone-dependent fear conditioning induced by a single training trial. In addition, the relationship between FOS levels and acquisition of conditioned fear was studied in mice preexposed to context or to context and shock before conditioning. This habituation to context or context paired with shock was performed by monitoring FOS production to determine the brain structures activated by individual Cs and Us as well as the degree of convergence of Cs and Us within a particular forebrain area.

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Correspondence should be addressed to Jelena Radulovic, Max Planck Institute for Experimental Medicine, Department for Molecular Neuroendocrinology, Hermann-Rein-Strasse 3, 37075 Goettingen, Germany.

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EXPERIMENTAL DESIGN

(n = 162)

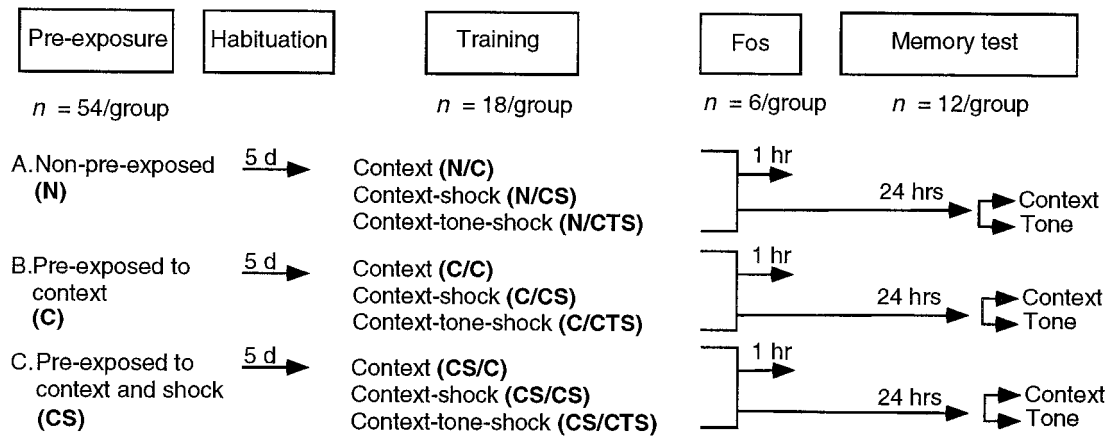


Figure 1. Experimental design.

MATERIALS AND METHODS

Animals. Eight-week-old male inbred C57BL/6N mice, obtained from Charles River (Sulzfeld, Germany), were used in the experiments. The mice were individually housed in macrolon cages according to the recommendations of the Society for Laboratory Animal Science (Germany). Standard pelleted diet and water were offered *ad libitum*. Mice were kept in mouse “hotels” 5 d before the beginning of the experiments. The mouse hotel and the entire fear-conditioning equipment were located in the same room to prevent exposure to novelty and transport of mice after the training or before the testing trials. The mouse hotel was located within a soundproof, nontransparent wooden enclosure equipped with its own air exchange system (air exchange, 60 m³/hr). In the mouse hotels, the temperature was maintained at 22 ± 1°C, the humidity was kept at 55 ± 10%, and the 12 hr light/dark cycle was 7 A.M. to 7 P.M.

Apparatus. As described previously (Radulovic et al., 1998; Stiedl and Spiess, 1997), the experiments were performed by a computerized fear-conditioning system purchased from TSE (Bad Homburg, Germany). The computer was connected to a control unit containing a shock and a tone generator. Training took place in an apparatus consisting of a box [58 cm (length) × 30 cm (width) × 27 cm (height)] with a gray interior and a 12 V light at the ceiling.

The conditioning and preexposure context consisted of a plexiglass chamber [35 cm (length) × 20 cm (width) × 20 cm (height)] placed on a removable shock grid made of stainless steel rods (diameter 4 mm; bars spaced 0.9 cm apart). The shock grid was connected to a shocker-scrambler unit delivering shocks of defined duration and intensity. The shock grid, the floor below the shock grid, and the chamber were cleaned with 70% aqueous ethanol before each individual mouse entered the chamber.

The pulsed auditory signal (five counts per second, 10 kHz, 75 dB sound pressure level) was presented for 30 sec by a high-frequency loudspeaker (Conrad, KT-25-DT).

The shock (0.7 mA, 2 sec, constant) was delivered through a stainless steel floor grid.

The novel context consisted of a Plexiglas chamber [35 cm (length) × 20 cm (width) × 20 cm (height)] that was exposed to 20 V light, did not have a rod floor, and was divided in half by inserting a clear plastic panel connecting the diagonal corners. The chamber was washed with 1% acetic acid before each individual mouse entered the chamber.

Fear conditioning. Nine experimental groups of mice were used in the experiments (Fig. 1). Three groups were set up for the preexposure procedures. Nonpreexposed mice (N) were habituated to handling and to the environmental stimuli of the experimental room for 3 min/d for 6 consecutive d. Two other groups of mice were preexposed either 3 min to the context (C) or 3 min to the context and a 2 sec shock (Cs) and then habituated as described above for 5 consecutive d. The habituation procedure was introduced to completely familiarize the mice with the

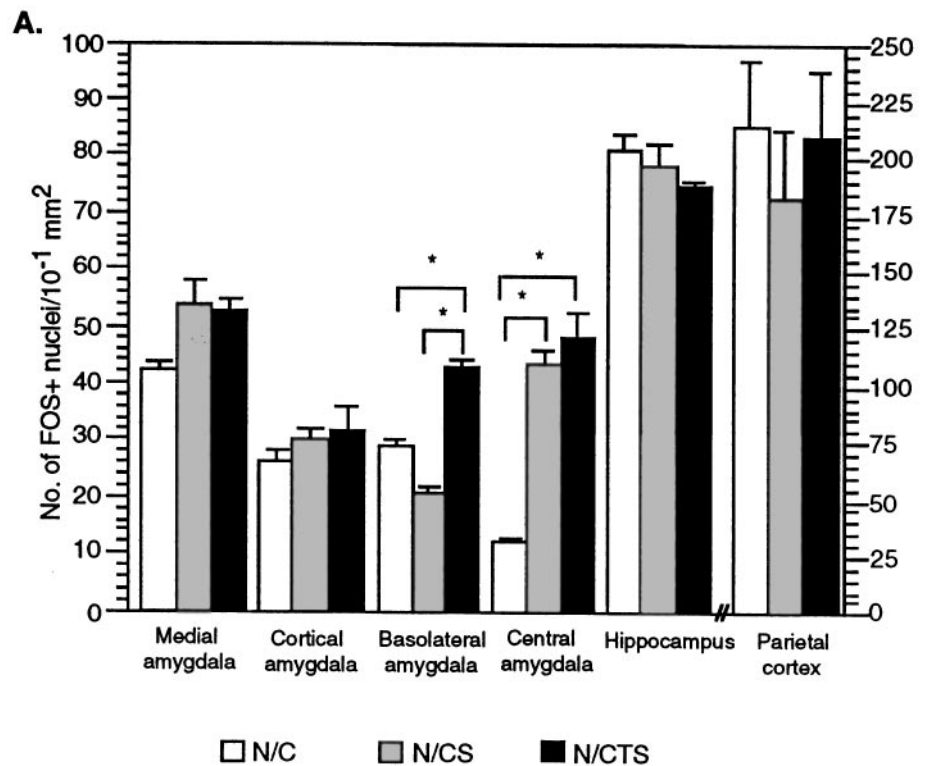
stimuli of the experimental room and thus prevent any interference of uncontrolled novel stimuli during the experiments. In addition, this procedure markedly reduced generalization of fear response to stimuli other than the Cs in mice of the Cs preexposure group.

On the training day, the N, C, and Cs preexposed mice were subjected to three additional training conditions. Mice of each group were exposed for 3 min to the context (N/C, C/C, and Cs/C groups), or 3 min context followed by a 2 sec shock (N/Cs, C/Cs, and Cs/Cs groups), or 3 min context, 30 sec tone, and 2 sec shock (N/CTS, C/CTS, and Cs/CTS groups). All preexposure and training procedures took place in the same context.

The contextual memory test was performed 24 hr after the training so that 12 mice of each experimental group were placed in the preexposure/conditioning context for 3 min. The tone-dependent memory test was performed 2 hr later, by exposure of the same mice to a novel context (3 min) followed by the tone used for conditioning (3 min). Freezing, defined as the lack of movement except for respiration and heart beat, was assessed every 10 sec over 18 intervals by a trained observer who was unaware of the experimental design. The data were converted to the percentage of samples scored as freezing.

FOS immunohistochemistry. One hour after the training, six mice of each group and four naive mice were anesthetized with ketamine (130 mg/gm body weight) and xylazine (13 mg/gm body weight) in saline (0.01 ml/gm, i.p.) and then transcardially perfused with ice-cold phosphate buffer (0.1 M), pH 7.4, followed by 4% paraformaldehyde in phosphate buffer, pH 7.4 (150 ml/mouse). Brains were post-fixed for 48 hr in the same fixative and then immersed for 24 hr each in 10, 20, and 30% sucrose in PBS. After the tissue was frozen by liquid nitrogen, 50- μ m-thick coronal sections were cut on the cryostat. Every fifth section was collected, starting from the olfactory bulb to the end of the hippocampus. Twelve-well plates (Corning, Corning, NY) were used for performing immunocytochemical staining of free-floating sections as described previously (Milanovic et al., 1998). After elimination of endogenous peroxidase activity by 1% H₂O₂ in methanol for 15 min, the sections were saturated with 5% goat serum and 0.3% Triton X-100 in 0.01 M PBS, and then incubated with rabbit anti-FOS antibody (Oncogene Science), 1:20,000 dilution for 48 hr at +4°C. Subsequently, the sections were washed and incubated at room temperature with biotinylated goat anti-rabbit antibody followed by the ABC complex (Vector ABC kit, Vector Laboratories, Burlingame, CA). For visualization, DAB was used as chromogen (Sigma fast tablet set). The sections were mounted, dehydrated, and coverslipped with Eukitt. The specificity of immunostaining was confirmed on sections that were incubated with FOS antibody preabsorbed overnight at +4°C with appropriate synthetic antigenic peptide in tenfold excess over the amount of antibody (Oncogene Science).

Quantification and data analysis. All sections from the olfactory bulb to the end of the hippocampus were analyzed qualitatively. FOS-positive



B.

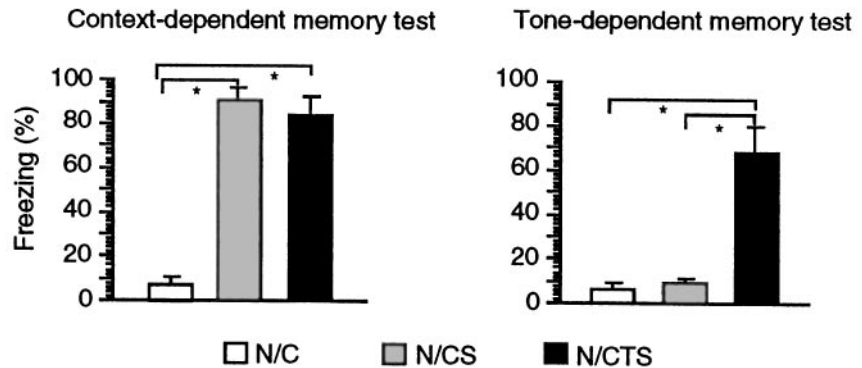


Figure 2. *A*, Fos production in the forebrain of N/C, N/Cs, and N/CTS groups 1 hr after training. Data represent mean \pm SE of six mice/group. *B*, Freezing behavior of the N/C, N/Cs, and N/CTS groups during the context- and tone-dependent memory test 24 hr after the training. Data represent mean \pm SE of 12 mice/group. Statistically significant differences: * $p < 0.01$.

cells were counted in selected forebrain areas with a Macintosh-based image analysis system (NIH Image), as described previously (Pomoni et al., 1997). Nuclei were counted individually and expressed as number of FOS-positive nuclei per 0.1 mm^2 . The anteroposterior (AP) coordinates of sections (Franklin and Paxinos, 1997) included for detailed analysis were as follows: AP -1.22 , medial nucleus of the amygdala; AP -1.34 central, cortical, and basolateral nucleus of the amygdala, CA1 region of the hippocampus and parietal (somatosensory) cortex. The counting was performed in an area of the same shape and size for each brain region. Statistical analysis of behavioral and immunohistochemical data were performed by ANOVA with preexposure and training as a group factor followed by the Bonferroni–Dunn test for *post hoc* comparisons. The results are presented as mean \pm SE.

RESULTS

FOS production after training and behavior during the memory test of nonpreexposed mice

After the training, FOS production of all nonpreexposed mice (N/C, N/Cs, and N/CTS groups) was observed throughout numer-

ous cortical, limbic, diencephalic, and mesencephalic areas. Exposure to context only (N/C group) significantly increased FOS production in the basolateral nucleus of the amygdala ($t_{(1,8)} = 10.55$, $p < 0.001$), hippocampus ($t_{(1,8)} = 6.84$, $p < 0.001$), and parietal cortex ($t_{(1,8)} = 10.64$, $p < 0.001$), in comparison with naive mice. Statistically significant group differences were determined within the central and basolateral nuclei of the amygdala (Figs. 2*A*, 3). In the central nucleus of the amygdala, mice of the N/Cs and N/CTS groups produced significantly more FOS ($F_{(8,45)} = 19.824$, $p < 0.001$) than mice of the N/C group. In mice of the N/CTS group, the number of FOS-positive nuclei in the basolateral amygdala was significantly higher ($F_{(8,45)} = 49.210$, $p < 0.001$) when compared with the mice of the N/C and N/Cs groups (Figs. 2*A*, 3). In the other forebrain areas examined for FOS production, no group differences were detected. It should be mentioned, however, that FOS production was also detected in

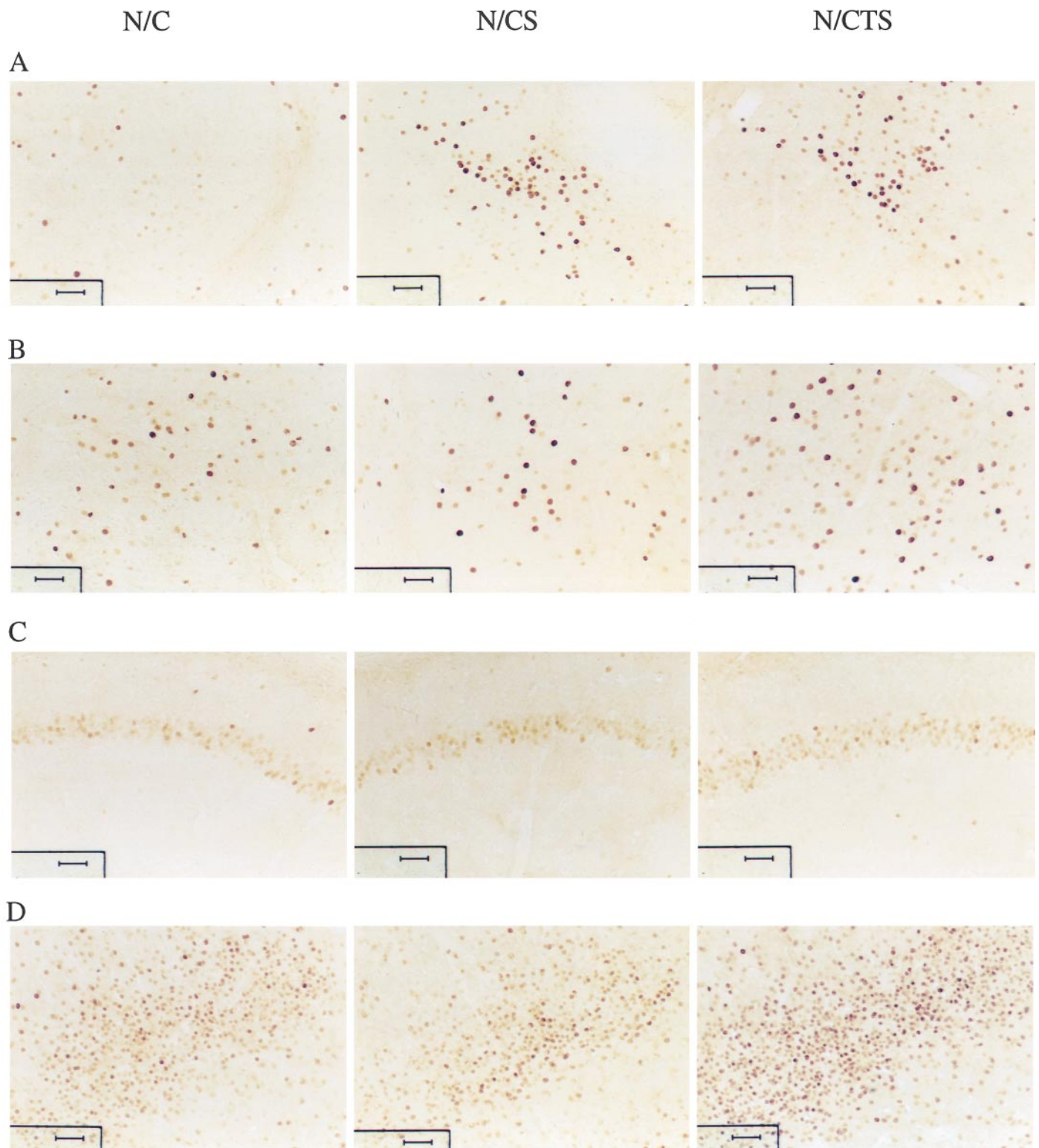


Figure 3. Fos production in the central amygdala (*A*), basolateral amygdala (*B*), hippocampus (*C*), and parietal cortex (*D*) of N/C, N/Cs, and N/CTS groups. Scale bar, 50 μ m.

numerous auditory areas, such as the inferior colliculus, medial geniculate nucleus, and auditory cortex of the N/CTS group. In additional experiments with mice exposed to context and tone without shock (data not presented), similar results were observed, except for the central amygdala, which showed Fos production on a low or undetectable level.

During the memory test, mice of the N/C group did not exhibit freezing behavior. Mice of the N/Cs group had high freezing levels during the context-dependent but not tone-dependent memory test, whereas mice of the N/CTS group responded with freezing during the context-dependent as well as the tone-dependent memory test (Fig. 2*B*).

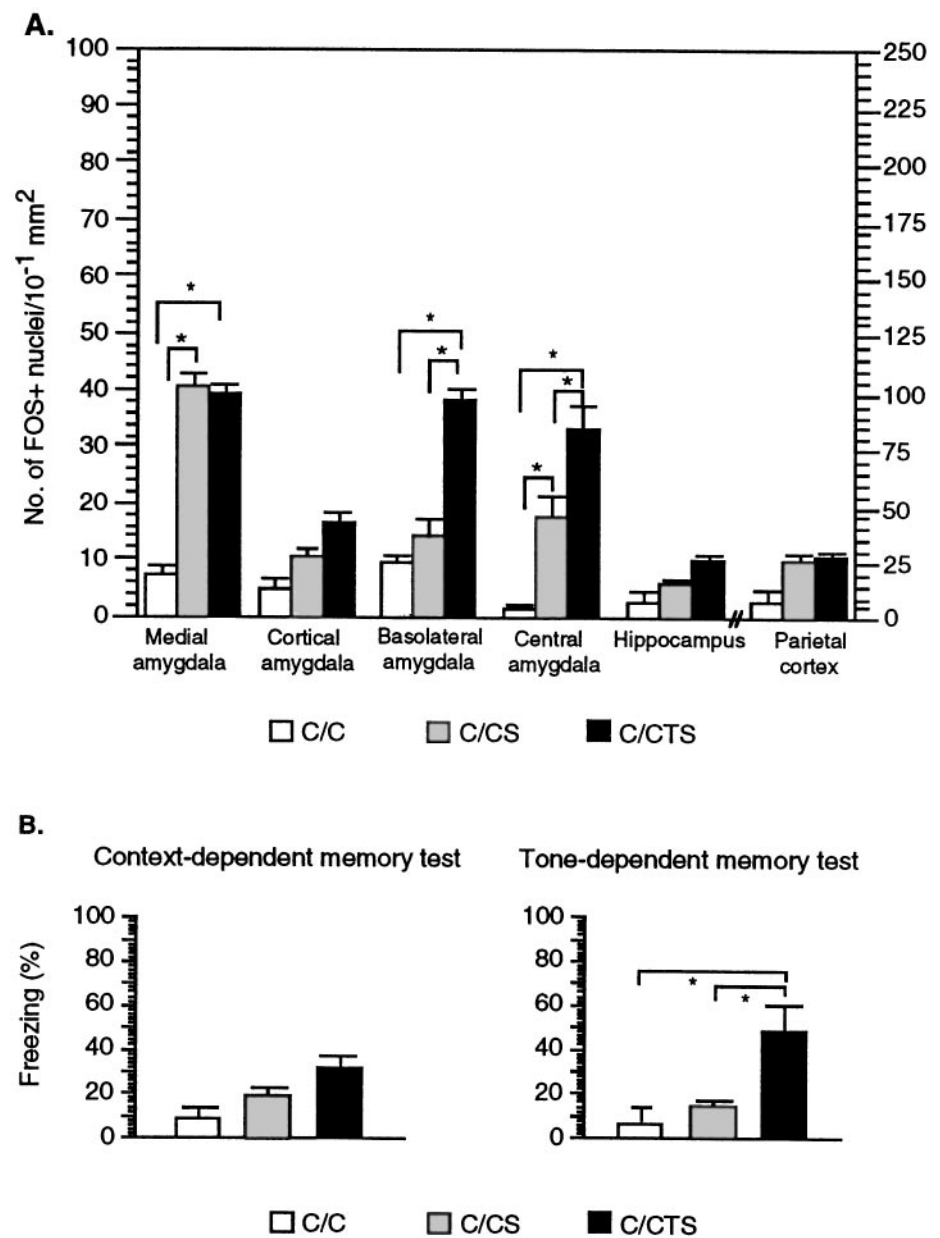


Figure 4. *A*, Fos production in the forebrain of C/C, C/CS, and C/CTS groups 1 hr after training. Data represent mean \pm SE of six mice/group. *B*, Freezing behavior of C/C, C/CS, and C/CTS groups during the context- and tone-dependent memory test 24 hr after the training. Data represent mean \pm SE of 12 mice/group. Statistically significant differences: * $p < 0.01$.

FOS production after training and behavior during the memory test of mice preexposed to context

FOS production in the C/C group of mice (Figs. 4*A*, 5) was significantly decreased in all tested areas ($p < 0.001$) when compared with the N/C group (Fig. 2*A*, Table 1). The observed values were similar to those found in naive mice (data not shown).

In mice of the C/CS group, FOS production was induced only in the central nucleus of the amygdala (Figs. 4*A*, 5) when compared with the C/C group ($p < 0.001$). However, the number of FOS-positive nuclei in this area was significantly lower than in the N/Cs ($p < 0.05$) or N/CTS group ($p < 0.05$) (Table 1).

In the C/CTS group, in comparison with the C/C and C/CS groups, the number of FOS-positive nuclei was significantly higher in the basolateral ($p < 0.001$) and central ($p < 0.01$) nuclei of the amygdala (Figs. 4*A*, 5). In those areas, FOS production did not differ from that of the N/CTS group. However, in all other brain areas, such as the hippocampus ($p < 0.001$), parietal cortex ($p < 0.001$), medial amygdala ($p < 0.001$), and cortical

amygdala ($p < 0.001$), the FOS levels were significantly lower than the FOS production of the N/CTS group.

Mice preexposed to context differed significantly from nonpreexposed mice in their ability to acquire context- and tone-dependent conditioned fear ($F_{(8,99)} = 419.629$, $p < 0.001$). Mice of the C/CS and C/CTS groups exhibited low freezing levels during the contextual memory test (Fig. 4*B*). The mice of the C/CTS group showed increased freezing during the tone-dependent memory test (Fig. 4*B*), but the freezing scores were significantly lower than those of the N/CTS mice subjected to the same training ($p < 0.001$).

FOS production after the training and behavior during the memory test of mice preexposed to context followed by shock

Cs preexposure of mice (Figs. 6*A*, 7) resulted in a significantly lower FOS production in all brain structures of the C/CS and C/CTS groups ($p < 0.001$) when compared with the N/C and N/CS

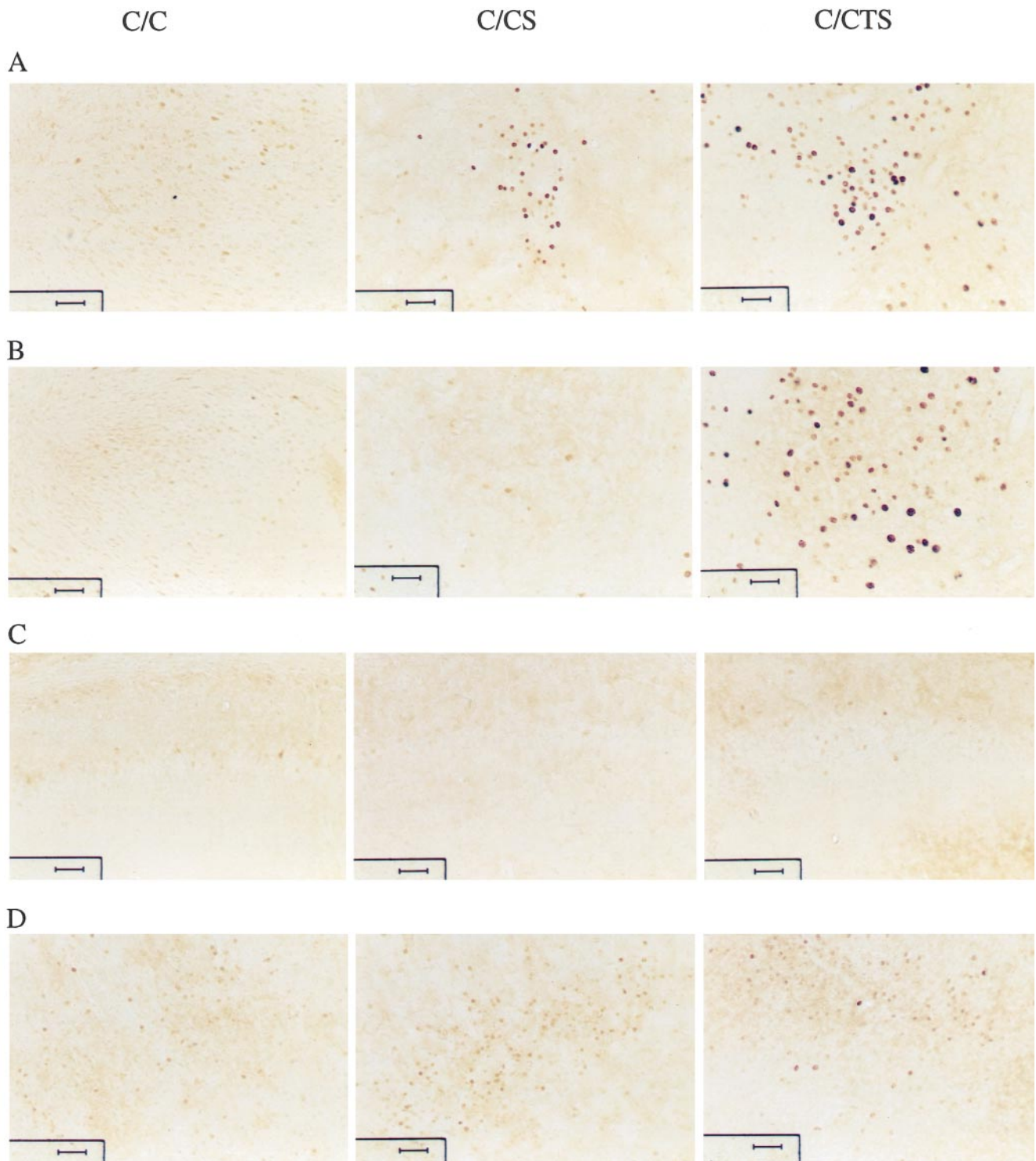


Figure 5. Fos production in the central amygdala (A), basolateral amygdala (B), hippocampus (C), and parietal cortex (D) of C/C, C/Cs, and C/CTS groups. Scale bar, 50 μ m.

groups (Table 1). In addition, mice of the Cs/Cs group had significantly lower ($p < 0.01$) Fos levels in the central nucleus of the amygdala than mice of the C/Cs group (Figs. 4A, 5, 6A, 7). In mice of the Cs/CTS group, significantly higher Fos production ($p < 0.001$) was detected only in the basolateral nucleus of the amygdala when compared with the Cs/C and Cs/Cs groups (Figs. 6A, 7). In this anatomical structure, Fos production of the

Cs/CTS group did not differ ($p = 0.403$) from the N/CTS group (Figs. 2A, 3, 4A, 5; Table 1), but in all other brain areas it was significantly lower ($p < 0.001$). Similar results were obtained in additional experiments with mice preexposed to context and reexposed to context and tone (data not shown).

Mice of the Cs/Cs and Cs/CTS groups acquired a strong context-dependent fear response that did not differ from the

Table 1. Significant differences in FOS production of groups subjected to the same training but different preexposure conditions

Group	Brain area					
	Medial amygdala	Cortical amygdala	Central amygdala	Basolateral amygdala	Hippocampus	Parietal cortex
<i>F</i> (8,45)	29.136	15.031	19.824	49.210	45.607	59.132
N/C						
C/C	***	***		***	***	***
Cs/C						
N/Cs	***	***	*	***	***	***
C/Cs	***	***	**	***	***	***
Cs/Cs						
N/CTS	***	***	***		***	***
C/CTS	***	***	***		***	***
Cs/CTS						

Statistically significant differences determined by a multivariate ANOVA: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

response of the N/Cs and N/CTS groups. However, tone-dependent conditioned fear was significantly reduced ($F_{(8,99)} = 419.629$, $p < 0.001$) in the Cs/CTS group (Fig. 6B) when compared with the N/CTS and C/CTS groups.

DISCUSSION

Widespread FOS production of nonpreexposed mice was observed in numerous brain areas, including the nuclei of the amygdala, hippocampus, and parietal cortex, in agreement with previous mapping studies (Handa et al., 1993; Papa et al., 1993). These data were interpreted as the response of animals to novel environmental stimuli. The observed differences between the N/C, N/Cs, and N/CTS groups confirmed and extended our previous results (Milanovic et al., 1998) indicating that the shock used as a Us in the N/Cs and N/CTS groups elicited marked FOS production in the central nucleus of the amygdala, whereas exposure to a tone as a novel Cs in the N/CTS group resulted in high FOS levels in the basolateral nucleus of the amygdala.

Repeated exposure of mice to the same Cs (C/C group) or Cs/Us (Cs/Cs group) resulted in a significant decrease of FOS levels and thus demonstrated that reduction of FOS production occurred after nonassociative as well as associative learning and that it was not affected by the emotional state of the mice as suggested previously (Sandner et al., 1993). These results contrasted with the observation that conditioned fear elicits c-fos mRNA and FOS production in the limbic system on reexposure of mice to a Cs (Campeau et al., 1991; Pezzone et al., 1992; Smith et al., 1992; Beck and Fibiger, 1995). It is difficult to explain the observed discrepancy. On the basis of our own experience, novel stimuli accidentally used before the training may have to be considered. We were only able to prevent this type of interference by extensive habituation of the mice to the training environment, the experimenter, and the lack of any transport before handling. Our results also contrasted with the observation that specific brain areas express c-fos mRNA after learning in instrumental paradigms (Castro-Alamancos et al., 1992; Bertaina and Destrade, 1995; Hess et al., 1995; Kleim et al., 1996). This discrepancy may be attributable to the differences between clas-

sical and operant conditioning and primarily attributable to the fact that animals do not need to perform a particular task during classical conditioning.

The data obtained from the preexposure experiments provided further evidence that novelty represented the major factor triggering FOS production. Therefore, it was possible to analyze the brain areas activated by individual novel Cs and Us. The results demonstrated that the strongest FOS production was observed after exposure of mice to novel contextual stimuli (N/C group), resulting in high FOS levels in the parietal cortex, the hippocampus, and all nuclei of the amygdala except for the central nucleus. When shock was introduced as a novel stimulus (C/Cs group), FOS production was induced only in the central nucleus of the amygdala, whereas exposure to a tone as a novel stimulus (Cs/Cs group) resulted in FOS production in the basolateral nucleus. These results indicated that the shock does not converge with the contextual and auditory stimuli within the cortical, hippocampal, and amygdaloid brain areas. In contrast, Cs/Us convergence at the hippocampal level was demonstrated during trace conditioning of the eyeblink response with the finding that both Us and Cs altered the neural activity of the CA1 hippocampal area (Mcechron and Disterhoft, 1997). The observed discrepancy may be attributable to different neural pathways mediating conditioned fear and conditioned eyeblink responses.

Although FOS production in the central nucleus of the amygdala was induced only by shock (C/Cs group) and not by context or tone, the FOS level within this nucleus was significantly enhanced when shock used as a novel Us was paired with novel contextual (N/Cs group) or auditory (C/CTS group) stimuli. It appears therefore that brain areas activated by a novel Cs have the capacity to facilitate the responsiveness of the central amygdala to a Us paired with the Cs. This interaction seems to occur only when both the Cs and Us are novel, because in the Cs/CTS group of mice, which were twice exposed to context and shock, the novel tone could not enhance FOS production in the central amygdala.

The contextual and auditory Cs used in the present experiments mainly elicited FOS production in different forebrain areas. An exception was the basolateral nucleus of the amygdala, where both Cs induced FOS. Although the highest FOS level in this area was found when novel contextual and auditory stimuli were applied simultaneously (N/CTS group), each Cs applied individually (N/C and Cs/CTS groups) also elicited FOS production, thereby suggesting that contextual and phasic Cs converge within the basolateral amygdala. This result is consistent with the finding that the basolateral amygdala (Kim and Fanselow, 1992) is required for both context- and tone-dependent fear conditioning.

The series of behavioral studies performed after different preexposure conditions strongly suggested a relationship between FOS production after the training and the learning efficiency monitored during the memory test. High FOS levels detected throughout the forebrain of nonpreexposed mice paralleled their ability to acquire strong contextual (N/Cs and N/CTS groups) and tone-dependent freezing (N/CTS group). These findings are in agreement with results pointing to significant roles of the amygdala (Helmstetter, 1992; Kim and Fanselow, 1992; Fanselow et al., 1994), hippocampus (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Chen et al., 1996), and parietal cortex (Thinus et al., 1996) in acquisition of context- and tone-dependent freezing as well as of other autonomic responses reflecting fear (Roosendaal et al., 1991). Accordingly, C and Cs preexposure resulted in reduction of FOS production in certain forebrain areas as well as in impaired acquisition of conditioned fear.

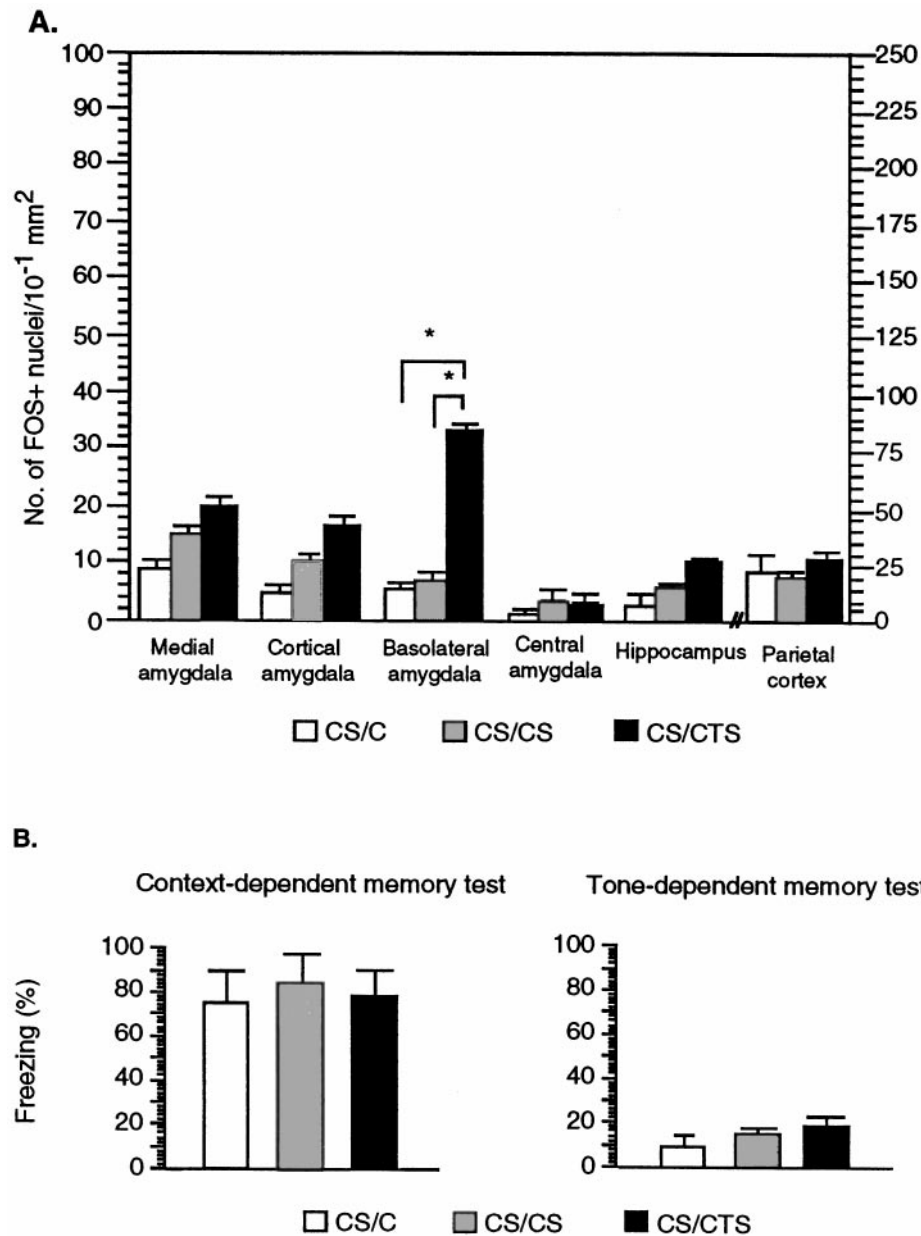


Figure 6. *A*, Fos production in the forebrain of Cs/C, Cs/CS, and Cs/CTS groups 1 hr after training. Data represent mean \pm SE of six mice/group. *B*, Freezing behavior of Cs/C, Cs/CS, and Cs/CTS groups during the context- and tone-dependent memory test 24 hr after the training. Data represent mean \pm SE of 12 mice/group. Statistically significant differences: * $p < 0.01$.

Significant decrease of contextual freezing of the C/Cs and C/CTS groups paralleled the low FOS level in the hippocampus, parietal cortex, and cortical and medial nuclei of the amygdala. Thus it appeared that activation of the central amygdala elicited by the Us after contextual preexposure was not sufficient for acquisition of context-dependent freezing. The tone-dependent freezing response of the C/CTS group was also significantly reduced, but to a lesser extent, implying that simultaneous activation of the basolateral and central amygdala may be sufficient for acquisition of tone-dependent freezing. This finding is in agreement with the observation that the involvement of hippocampus is not required for acquisition of tone-dependent fear (Selden et al., 1991; Kim and Fanselow, 1992; Phillips and LeDoux, 1992). The significance of simultaneous FOS production in the basolateral and central amygdala for tone-dependent fear conditioning was further supported by the finding that the Cs/CTS group, in which FOS production was detected only in the

basolateral but not central amygdala, acquired very weak tone-dependent freezing. On the basis of these findings it was concluded that synchronous FOS production, followed most probably by activation of specific molecular cascades (Morgan and Curran, 1991), in defined brain areas is accompanied by associative learning involving novel stimuli. Interestingly, the novel neutral stimuli used as Cs exhibited a much stronger capacity to induce the production of the FOS protein than the novel aversive stimulus used as Us.

Activation of certain brain areas during latent inhibition has been reported previously using a conditioned emotional response procedure and using FOS as a marker of neural activity. Latent inhibition was induced by preexposure of rats to both contextual and auditory Cs and resulted in reduction of the FOS production in most of the brain areas, except for the nucleus accumbens, dentate gyrus, and subiculum, where an increase was detected (Sotty et al., 1996). In those and other areas, however, we could not find any increase of the FOS protein after reexposure to the

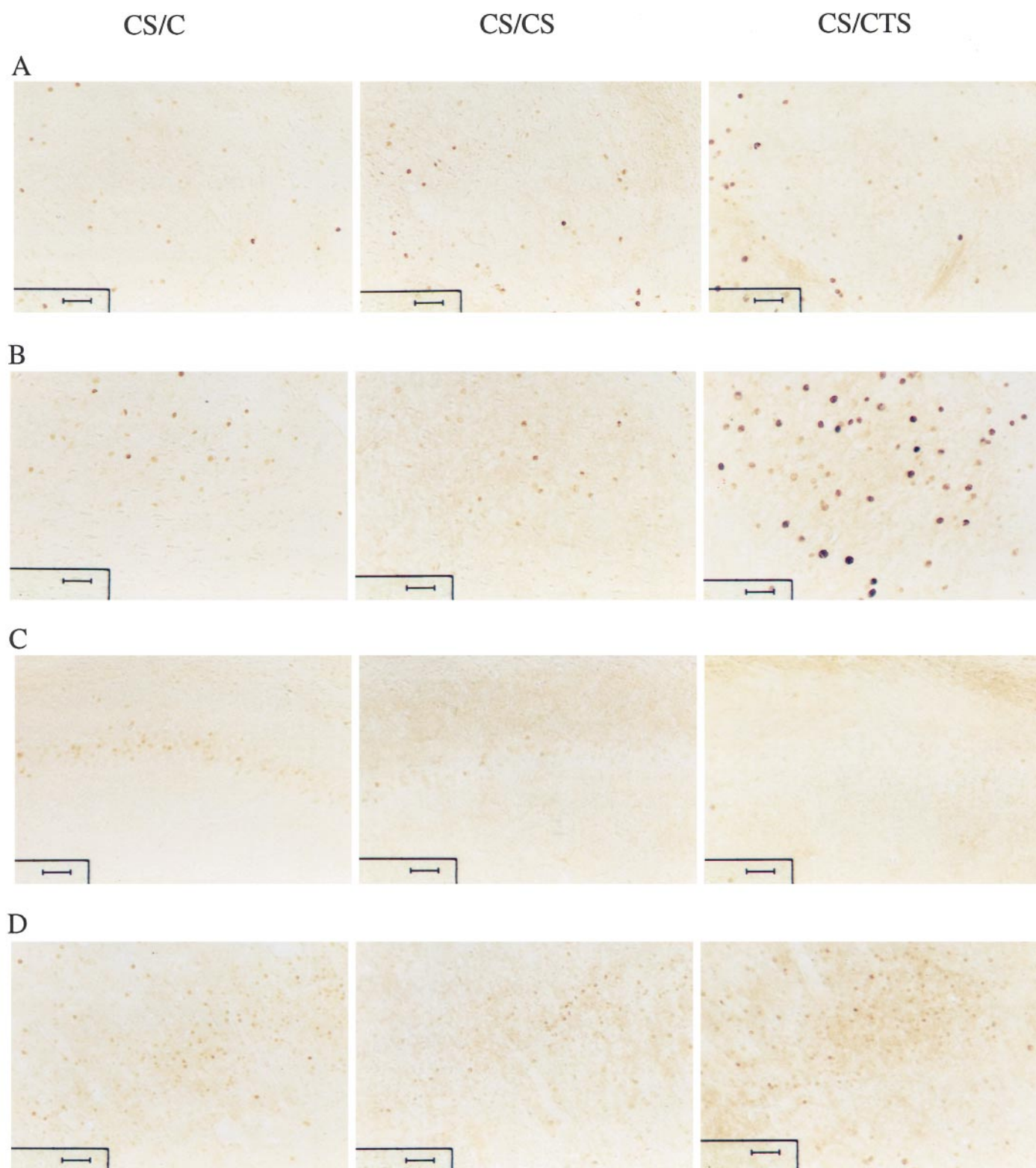


Figure 7. FOS production in the central amygdala (*A*), basolateral amygdala (*B*), hippocampus (*C*), and parietal cortex (*D*) of Cs/C, Cs/Cs, and Cs/CTS groups. Scale bar, 50 μ m.

same Cs and Us (data not shown). We assume that this discrepancy may be attributable to numerous differences in the experimental conditions, such as the type of the learning procedure, sequence and number of stimuli presentations during the preex-

posure and training, number of training trials, interference of novel stimuli, and the time point of FOS immunodetection.

So far, the phenomenon of latent inhibition has been explained mainly by decreased attentional processes during encounter of

stimuli. A major role has been attributed to various neurotransmitter systems (Dunn et al., 1993; Baxter et al., 1997). The present findings extend these observations, suggesting that reduction of FOS may reflect the decrease of Cs processing underlying latent inhibition. The same molecular changes seem to be involved in the Us preexposure effect, thus providing a relatively simple molecular basis for a phenomenon that has been described by numerous adaptational (Taylor, 1956; Kamin, 1961; Mis and Moore, 1973), associative (Tomie, 1976), and cognitive (Baker and Mackintosh, 1979) psychological models. Taking into account that a causal relationship between FOS production and learning has been demonstrated recently, as mentioned above (Mileusnic et al., 1996; Swank et al., 1996), the results obtained in this study suggested that learning impairments induced by Cs and Cs/Us preexposure might have been caused by downregulation of FOS production in specific brain areas. This explanation would be consistent with the hypothesis that reduced processing of Cs and Us, which occurs when they are learned as consistent predictors of certain events, prevents subsequent association of the same Cs and Us with other events.

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