

Contextual Fear Conditioning Is Associated With Lateralized Expression of the Immediate Early Gene *c-fos* in the Central and Basolateral Amygdalar Nuclei

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Fos, the protein product of the immediate early gene *c-fos*, was used to map functional circuitry underlying contextual conditioned fear. Male rats were given footshocks in a distinctive context and later tested using freezing as the behavioral measure and compared with no-shock and no-retention-test control groups. An increased number of Fos-immunoreactive neurons was found in the lateral part of the central nucleus and in the anterior basolateral and lateral amygdalar nuclei in the brains of the conditioned-fear group compared with controls. Further, a greater number of Fos-immunoreactive neurons was observed in the right central and anterior basolateral nuclei compared with the number of labeled neurons in these structures on the left.

Fear conditioning is a form of learning that has been widely used as a model for studying the neural substrates involved in emotional learning and memory (Davis, 1992; Fanselow & Kim, 1994; Kapp, Whalen, Supple, & Pascoe, 1992; LeDoux, 2000; Maren, 2001). In this model, an initially neutral stimulus such as a tone, light, or context of conditioning chamber (conditioned stimulus; CS) comes to elicit conditioned-fear responses after being paired with an aversive unconditioned stimulus (US) such as footshock. Conditioned-fear responses involve a complex, highly coordinated set of autonomic, neuroendocrine, and species-specific behavioral responses that include two commonly used measures of fear: somatomotor immobility (i.e., freezing) and modulation of acoustic startle reflex (for reviews, see Davis, 1992; LeDoux, 2000).

Accumulating evidence from behavioral and anatomical studies has helped delineate critical components of the fear-conditioning circuit within the amygdala that are important for learning and expressing conditioned fear, respectively (for reviews, see Fanselow & LeDoux, 1999; LeDoux, 2000; but also see Cahill, Weinberger, Roozendaal, & McGaugh, 1999). In addition, a number of studies examined the involvement of the amygdala (Beck & Fibiger, 1995; Campeau, Falls, Cullinan, Helmreich, Davis, & Watson, 1997; Campeau, Hayward, Hope, Rosen, Nestler, & Davis, 1991; Milanovic et al., 1998; Pezzone, Lee, Hoffman,

& Rabin, 1992; Radulovic, Kammermeier, & Spiess, 1998; Rosen, Fanselow, Young, Sitcoske, & Maren, 1998; Smith, Banerjee, Gold, & Glowa, 1992) in conditioned-fear processing using an immediate early gene *c-fos*, or its protein product Fos, as a marker for neuronal activation (Ceccatelli, Villar, Goldstein, & Hokfelt, 1989; Dragunow & Faull, 1989; Morgan & Curran, 1991). However, these studies produced conflicting results. One set of studies showed that Fos production in the amygdala is not correlated with the conditioned-fear responses (Campeau et al., 1997; Radulovic et al., 1998; Rosen et al., 1998; Smith et al., 1992), whereas another set of studies showed increased Fos production in the amygdala after exposure to the conditioned stimulus that was previously paired with an aversive event (Beck & Fibiger, 1995; Campeau et al., 1991; Milanovic et al., 1998; Pezzone et al., 1992). Furthermore, studies that showed *c-fos* activation within the amygdala after reexposure to the CS are inconsistent in regard to the exact region of the amygdala activated. Pezzone et al. (1992) as well as Milanovic and colleagues (1998) found conditioned fear associated Fos protein expression in the medial nucleus of the amygdala, whereas Beck and Fibiger (1995) found an increase in Fos protein expression in the central, basolateral, and basomedial amygdalar nuclei.

The discrepancies in the above mentioned studies might be due in part to differences in procedures used and in part to the complex organization of amygdalar areas that are components of the conditioned-fear circuitry. These amygdalar areas display distinct connectional features (Swanson & Petrovich, 1998) and are likely to play different roles in fear conditioning. Recent evidence suggests that the lateral (LA) and/or anterior and posterior basolateral (BLAa and BLAp, respectively) nuclei of the amygdala and the central nucleus (CEA) are critical for the learning and expression, respectively, of conditioned-fear responses (for reviews, see Fanselow & LeDoux, 1999; LeDoux, 2000; Maren, 2001; Savander, Go, LeDoux, & Pitkänen, 1995; but see Killcross, Robbins, & Everitt, 1997, for a differing view). The CEA has three structurally distinct parts: medial (CEAm), lateral (CEAl), and capsular (CEAc; Cassell, Gray, & Kiss, 1986; McDonald, 1982),

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and the main output to the brainstem regions that mediate autonomic and behavioral aspects of conditioned-fear responses originates in the CEAm (Hopkins & Holstege, 1978; Rizvi, Ennis, Behbehani, & Shipley, 1991; Schwaber, Kapp, Higgins, & Rapp, 1982).

Thus, we sought to provide a more anatomically detailed and quantitative map of Fos expression within the amygdala elicited by the contextual CS that had previously been paired with footshocks. Specifically, we examined Fos distribution on each side of the brain separately, within the LA and each subregion of the BLA and CEA.

Method

Subjects

The subjects were 36 experimentally naive, young adult, male rats of Sprague-Dawley descent (250–300 g) obtained from a commercial supplier (Harlan Sprague Dawley, Indianapolis, IN). Animals were individually housed with ad-lib access to food and water and maintained in a climate-controlled vivarium on a 12-hr light–dark cycle. All experiments were conducted between 6:00 and 10:00 (light cycle). Before the experiment, animals were assigned randomly to either trained, control, or shock-control groups. Prior to conditioning, for 7 days, the animals were transported to the conditioning room and held daily for 5 min for adaptation.

Behavioral Apparatus

Conditioning and contextual fear testing were performed in a modular operant observation chamber (27 × 28 × 30.5 cm; Coulbourn Instruments,

Allentown, PA) that was situated in a brightly lit and isolated room. The front and back of the chamber were constructed of clear acrylic plastic, and the top and sides were constructed of aluminum. The floor of the chamber consisted of 16 stainless steel rods (4-mm diameter) spaced 17 mm apart (center to center) that were connected to a shock generator (Precision Controlled Animal Shocker, Coulbourn Instruments, Allentown, PA). The delivery of the footshock US was controlled by L2T2 Operant Control Software (Version 4.0; Coulbourn Instruments, Allentown, PA). An 80-dB white noise supplied the background noise. Prior to conditioning and fear testing, the chamber was cleaned with 5% ammonium hydroxide solution.

Fear Conditioning

Four groups ($n = 9$ per group) of animals were used in this experiment (see Figure 1): trained groups (conditioned-fear groups; A and B) and control groups (C and D). The trained groups were trained for 2 days (Day 1 and Day 2). On each training day, the rats were transported to the conditioning room and placed in the experimental chamber. Three minutes after being placed in the chamber, the rats received three unsignaled footshocks (1 mA; 1 s; 60-s intertrial interval). At 60 s after the third shock, the rats were immediately returned to their home cages. On Day 3, the animals were left undisturbed to allow for possible shock-training-induced changes in *c-fos* expression to return to baseline. On Day 4, fear conditioning to the context of the conditioning chamber was assessed by returning the rats to the conditioning chamber and measuring freezing behavior (defined as the lack of movement except that necessitated by respiration) during an 8-min (Group A) or 30-min (Group B) extinction test. The Trained Group B was tested for 30 min to determine if a longer exposure to the context increases *c-fos* activity because preliminary results with Trained Group A, which had an 8-min context test, showed virtually no

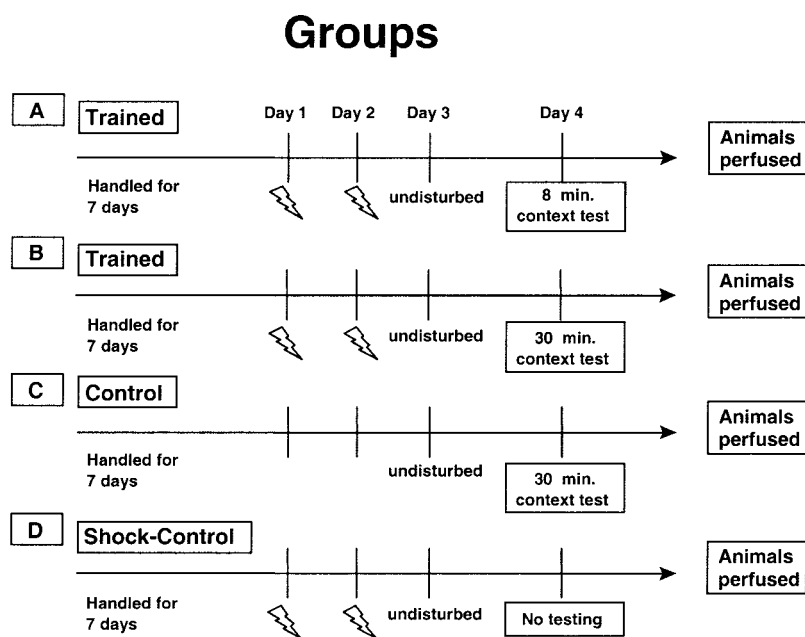


Figure 1. Experimental design. Animals in Groups A, B, and D received three footshocks per session (1 s, 1 mA; intertrial interval = 1 min) for 2 days (one session per day); no footshocks were administered to animals in Group C. Two days after the training session, animals in Group A were tested for 8 min, and animals in Groups B and C were tested for 30 min. Freezing was used as a behavioral measure of fear. Seventy-five minutes after the end of the tests on Day 4, all animals in Groups A, B, and C were perfused, and their brains were collected and pretreated for anatomical procedures. Group D was never tested; instead, animals were perfused at the time they would have been tested.

c-fos expression. After testing, the animals were taken back to their home cages.

Animals in Control Group C (no training) followed the same protocol as the trained group, except that the group received no footshocks; this group was controlled for animals' exposure to handling, transportation, and the training environment alone. Shock-Control Group D (training only) received the same training as Trained Groups A and B, including footshocks, except that they were never tested for fear conditioning to the context; instead, they were perfused at the time testing would have begun on Day 4. This group was important because it provided information about possible training-induced changes in Fos protein levels immediately prior to testing.

Behavioral Analysis

Freezing was assessed independently by two observers who scored blindly the behavior of each rat every 5th min during the 30-min testing period. In addition, each animal's movement (or immobility) was measured continuously by a 24-cell infrared activity sensory (L2T2 LabLinc System, Coulbourn Instruments, Allentown, PA) that was mounted on top of the experimental chamber by measuring the emitted infrared (13 nm) body heat image from the animal in the *x*, *y*, and *z* axes. Lee and Kim (1998) described this procedure in detail previously. Both measurements are presented as a percentage of total observations during the testing period. All data are represented as the means plus or minus the standard errors of measurement (see Figure 2).

Fos Immunohistochemistry

Exactly 75 min after the testing period ended, the animals were quickly and deeply anesthetized with pentobarbital and then perfused transcardially with 4% paraformaldehyde according to the protocol described elsewhere (Swanson & Simmons, 1989; Petrovich & Swanson, 1997). The brains were then collected and pretreated for anatomical procedures. Five animals were chosen randomly from each experimental group for anatomical procedures.

For histochemical analysis, frozen brains were cut on a sliding microtome into five adjacent series of 24- μ m-thick transverse sections. One

complete series of sections was processed to detect *c-fos* expression, using a standard immunohistochemical procedure. Briefly, the sections were processed with a rabbit antibody against Fos (48 hr, 4 °C; Oncogene Research Products, San Diego, CA; dilution 1:20,000) and a solution containing avidin-biotin-horseradish peroxidase (HRP) complex (ABC Elite Kit, Vector Laboratories, Burlingame, CA). Staining was obtained by processing the peroxidase histochemistry with a solution containing 0.05% diaminobenzidine and 0.01% hydrogen peroxide. The sections were then mounted on gelatin-coated slides, dehydrated, and coverslipped with DPX (Electronic Microscopy Sciences, Fort Washington, PA). An adjacent series was stained with thionin for cytoarchitectonic purposes.

Quantification and Data Analysis

Sections throughout the CEA, LA, and BLA were analyzed quantitatively following the parcelation and nomenclature of the rat brain used in Swanson's rat brain atlas (Swanson, 1998–1999). Every section was analyzed starting at Caudal Level 29 of the Swanson atlas to Rostral Level 26 for the CEAl and to Level 25 for the CEAc and CEAm (trained group, *n* = 5 for right and left CEAl, CEAc, and CEAm; control group, *n* = 4 for right CEAl, CEAc, and CEAm; and *n* = 5 for left CEAl, CEAc, and CEAm; shock control, *n* = 5 for all right and left nuclei). For the BLA, every other section was analyzed from rostral to caudal starting at Level 24 to Level 29 for the BLAa, 28–34 for BLAp, and from caudal to rostral starting from Level 32 to Level 28 for the LA (trained group, *n* = 5 for right LA, BLAa, and BLAp and for left LA and BLAa; *n* = 4 for left BLAp; control group, *n* = 4 for right LA, BLAa, and BLAp; *n* = 5 for left LA, BLAa, and BLAp; shock-control group, *n* = 5 for right and left LA, BLAa, and BLAp).

Photographs of the Fos-stained sections as well as their adjacent thionin-stained sections were acquired, stacked, and registered using NIH Image (Rasband, 2002). Borders were drawn on the thionin-stained sections, and counting was performed on the adjacent Fos-stained section in the area where the border was drawn. Rolling ball was used to remove the background on the Fos-stained sections. Density analysis was used to count the number of Fos-positive cells in various amygdalar cell groups. Right and left sides were analyzed for all cell groups. Statistical analysis of immunohistochemical data was performed by a three-way analysis of variance

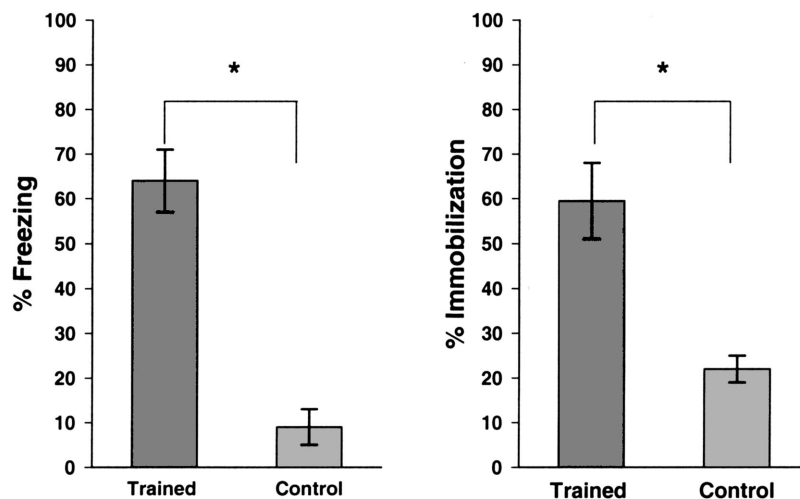


Figure 2. The animals' behavior during the 30-min context test was measured as percent freezing (left) and percent immobilization (right). Lack of movement (immobilization) is a less significant measure of conditioned fear in longer tests because in the second half of the testing period, animals in the control group do not move for reasons other than freezing (e.g., they are sleeping or resting). Freezing and immobilization are expressed as a mean (\pm SEM) percentage of total observations or total behavior, respectively, during the 30-min test period (*n* = 9 for all groups; only Groups B and C shown). * *p* < .05.

(ANOVA), with experimental group, cell group, and side as independent variables followed by the Tukey test for post hoc comparisons. All data are presented as means of the total number of cells per brain plus or minus standard errors of measurement.

Results

Behavior

For both trained groups (Group A: 8-min context test; Group B: 30-min context test; see Figure 1), the animals' behavior during the test period was measured. On average the trained groups displayed freezing behavior more than 60% of the time during the testing period, whereas the control group (Group C, see Figure 1) froze less than 10% of the time (see Figure 2; only Groups B and C shown). Animals from the shock-control group (Group D, see Figure 1) were not tested behaviorally because they were perfused at the time testing would have begun.

Immunohistochemistry

Fos production in the Trained Group A (8-min context test) was undetectable in any region of the CEA or BLA and so was not analyzed. All of the following comparisons between trained, control, and shock-control groups involve the Trained Group B (30-min context test).

CEA

Analysis of Fos production using a three-way ANOVA with variables of experimental group (trained, control, shock control), cell group (CEAl, CEAc, CEAm), and side (right and left) revealed a significant main effect of experimental group, $F(2, 71) = 37.0$, $p < .01$; cell group, $F(2, 71) = 18.0$, $p < .01$; and side, $F(1, 71) = 6.4$, $p < .05$, as well as significant Experimental Group \times Cell Group interaction, $F(4, 71) = 6.7$, $p < .01$; Experimental Group \times Side interaction, $F(2, 71) = 3.6$, $p < .05$; Cell Group \times Side interaction, $F(2, 71) = 4.2$, $p < .05$; and Experimental Group \times Cell Group \times Side interaction, $F(4, 71) = 2.8$, $p < .05$. Post hoc analysis (Tukey's honestly significant difference [HSD] unequal N) revealed that Fos production was significantly higher in the CEAl of the trained group (B) as compared with Fos production in the CEAl of the control and shock-control groups ($p < .01$ for each comparison; see Figure 3). There was no significant difference between the CEAl of the control and shock-control groups (see Figure 4A). Thus, increases in Fos production in the CEAl is specific to the group of rats that was exposed to the chamber (contextual CS) where footshocks were administered during training.

In the CEAc, there was no difference between trained group and control group, although there was a significant increase in the trained group as compared with the shock-control group ($p < .01$; data not shown). This suggests that Fos activation within the CEAc is not related to the contextual CS but rather to the handling procedures and transport that both trained and control groups experienced but that the shock-control group did not.

Separating the left and right sides of the brain revealed that the number of Fos-labeled neurons in the right CEAl of the trained group (see Figure 3B) was significantly higher than that in the left CEAl of the trained group (see Figure 3D), and it was also higher

than the number of Fos-stained neurons in the right and left CEAl of the control and shock-control groups (see Figures 3F, 3H, only right side shown; $p < .01$ for all comparisons, see Figure 4B). Furthermore, the number of Fos-stained neurons in the right CEAl was compared with the other parts of the CEA. Fos production was significantly higher in the right CEAl compared with the right or left CEAc and CEAm ($p < .01$ for all comparisons; see Figure 4C). In the control groups, there were no significant differences between right and left CEAl or between the different parts of the CEA (see Figure 4C). Thus, after exposure to the contextual CS, Fos production is increased specifically in one region of the central nucleus: the right CEAl.

LA and BLA

Analysis of Fos production using a three-way ANOVA with variables of experimental group (trained, control, shock-control), cell group (LA, BLAa, BLAp), and side (right and left) revealed a significant main effect of experimental group, $F(2, 68) = 32$, $p < .01$, and cell group, $F(2, 68) = 4.4$, $p < .05$, as well as a significant Group \times Side interaction, $F(2, 68) = 8.3$, $p < .01$. Post hoc analysis (Tukey's HSD unequal N) revealed that Fos production in the LA was significantly higher in the trained group than in both control groups ($p < .01$ for both comparisons; see Figure 5). There was no significant difference between the control groups (see Figure 7A). There was no difference in Fos production between the left and right LA of the trained groups.

In the BLAa (which corresponds in part to the magnocellular and intermediate divisions of Savander et al., 1995), Fos production was significantly higher in the trained group as compared with the control or shock-control groups ($p < .01$ for each comparison; see Figure 6). It is interesting to note that the number of Fos-stained neurons within the BLAa of the control group was significantly higher than in the shock-control group ($p < .01$; see Figure 7B), suggesting that, in addition to activation by the contextual CS (trained group), Fos production within the BLAa is also sensitive to the handling and transport that the control group was exposed to as compared with the shock-control group.

Separating the left and right sides revealed that Fos production in the right BLAa was significantly higher than Fos production in the left BLAa in the trained group. Furthermore, Fos production in the right BLAa of the trained group was significantly higher than it was in the right or left BLA of the control or shock-control groups ($p < .01$ for each comparison; see Figure 7C). In contrast to the BLAa, no differences were found in the number of Fos-stained neurons between the three groups (trained, control, and shock-control) in the BLAp (which corresponds in part to the parvocellular division of the basal nucleus of Savander et al., 1995).

Discussion

Two main results emerged from the present experiments. First, we found an increase in Fos protein levels, specifically in amygdalar regions that form parts of the fear conditioning circuit (LA, CEAl, and BLAa) after exposure to the context where the animal previously received a footshock. Second, we observed lateralization of conditioned-fear-associated Fos increases in the CEA and BLA. More neurons in the right CEAl

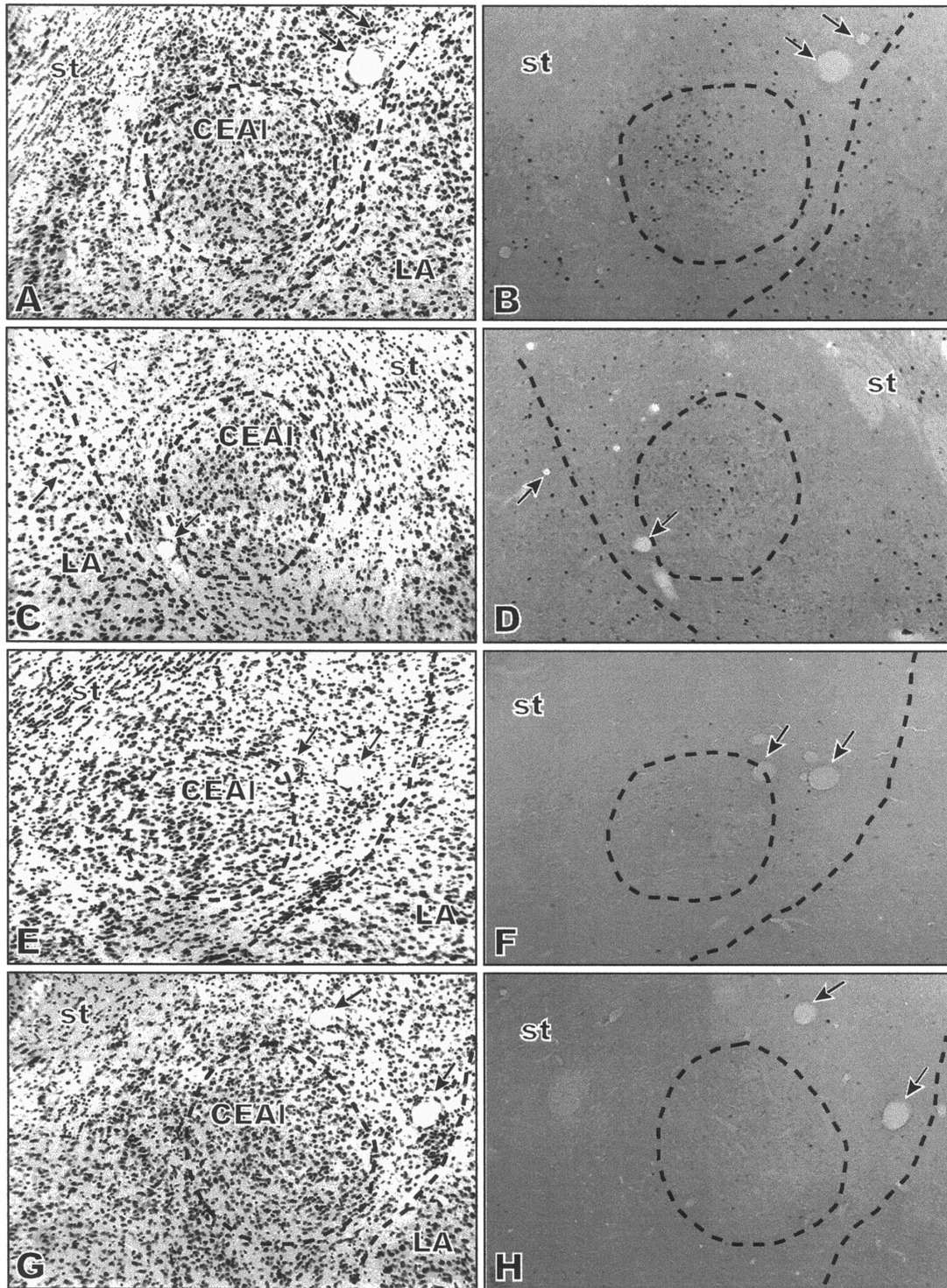


Figure 3. Brightfield photomicrographs of Nissl-stained (left) and Fos-stained (right) tissue in and around the central amygdalar nucleus (CEA). Right side of the brain (A, B) and left side of the brain (C, D) transverse sections from Trained Group B. Right side of the brain transverse sections from control group (E, F) and shock-control group (G, H). Arrows point to corresponding blood vessels in both photomicrographs. CEAI = CEA, lateral part; LA = lateral amygdalar nucleus; st = stria terminalis.

and BLAa showed Fos labeling after exposure to the contextual CS as compared with neurons in the left CEAl and BLAa, whereas conditioned-fear-associated Fos induction was bilateral in the LA.

The assumption that observed increases in Fos expression are directly related to or dependent on the elicitation of conditioned-fear by the contextual cue is supported by behavioral differences between the trained and control groups. Animals in the trained group display freezing behavior (a behavioral measure of fear) when exposed to the contextual chamber where they previously received footshocks, and they also show increased amygdalar Fos expression. In contrast, animals in the control group that never received footshock do not show the behavioral expression of fear when exposed to the experimental chamber and also showed low levels of Fos expression under these circumstances. These results are also consistent with our previous observation that increased enkephalin mRNA levels in the amygdala are associated with contextual CS (Petrovich, Scicli, Thompson, & Swanson, 2000).

The observed increases in Fos levels cannot be attributed to stress from handling and transport because all of the animals in the trained and control groups experienced the same procedure. The increases also cannot be attributed to the residual effects of training because the Fos levels were negligible at the time of testing in animals that previously experienced footshocks (shock-control group). However, our results do not speak to whether Fos activation is related to the expression of conditioned fear, to the retrieval of conditioned-fear memories, or to both. Amygdalar regions that show increased Fos levels in the present study are believed to be critical for both the acquisition and expression of conditioned fear (Fanselow & LeDoux, 1999; Killcross et al., 1997; Maren, 2001), although this view has been questioned (Cahill et al., 1999). Future research is needed to clarify the role played by Fos in conditioned fear.

The lack of detectable Fos induction in the group of animals that was exposed to the contextual CS for a short period of time (Group A), as contrasted with animals exposed to the same stimulus for longer time (Group B), could reflect a lack of sensitivity in the technique or a different time course for *c-fos* expression. As mentioned in the introduction, there are discrepancies in the literature about the occurrence and anatomical localization of changes in amygdalar Fos protein or *c-fos* mRNA levels after exposure to conditioned stress. Thus, differences in length of exposure to the CS could account for some discrepancies observed in earlier studies.

Our results are consistent with previous work that showed conditioned-fear-associated increases in amygdalar Fos levels (Beck & Fibiger, 1995; Campeau et al., 1991; Milanovic et al., 1998; Pezzone et al., 1992). However, there are some anatomical differences in our results and those reported earlier. We observed increased Fos production within the LA, CEAl, and BLAa, whereas earlier studies implicated the medial nucleus (Beck & Fibiger, 1995; Milanovic et al., 1998; Pezzone et al., 1992). As mentioned in the introduction, some studies showed no conditioned-fear-induced changes in amygdalar Fos protein levels, or *c-fos* gene expression. These discrepancies may be due to differences in experimental procedures, including variations in duration of exposure to the CS (see above), use of explicit versus contextual cues, number

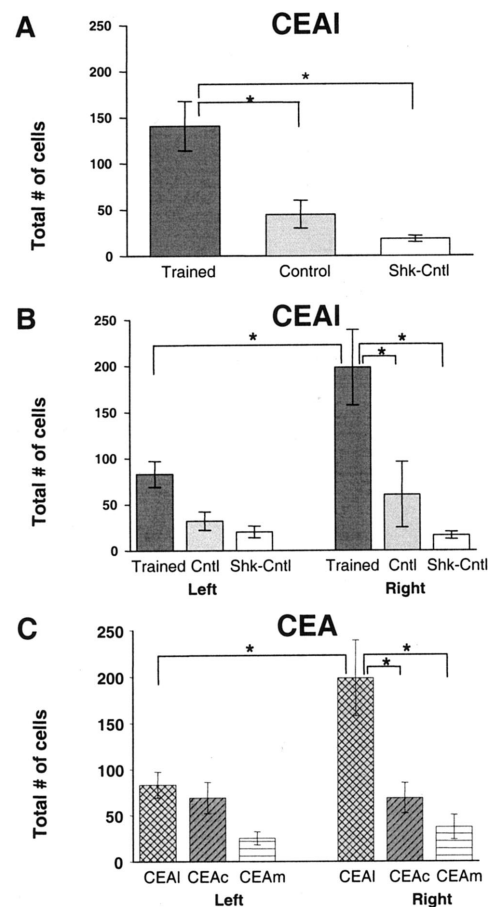


Figure 4. A: An increase in the number of Fos-immunoreactive neurons was found specifically in the CEAl of the trained group as compared with either the control or shock-control groups. B: The number of Fos-stained neurons in the left and right CEAl of the three groups. C: The number of Fos-stained neurons in the CEAl, CEAc, or CEAm on each side of the brain. CEA = central amygdalar nucleus; CEAc = CEA, capsular part; CEAm = CEA, medial part; CEAl = CEA, lateral part; Shk-cntl = shock-control. * $p < .01$.

of training trials with footshocks, or the time point for Fos detection. Clearly, further delineation of the mechanisms whereby stressors augment or fail to augment amygdalar *c-fos* expression is needed.

Finally, novelty is a powerful stimulus for Fos induction (Radulovic et al., 1998), and repeated exposure to the same stimulus blunts Fos responses (Chen & Herbert, 1995; Hess, Lynch, & Gall, 1995; Papa, Pellicano, Welzl, & Sadile, 1993). In our experimental design, the contextual CS that elicited amygdalar Fos production could not be regarded as a novel stimulus because both control and trained groups were exposed to it during the training phase. Furthermore, the expression of conditioned fear observed in the trained group of animals shows that these animals recognized the CS and remembered its association with the aversive event. Nevertheless, if the contextual CS that is presented without footshocks during the tests is regarded as novel because of the absence of footshocks, then Fos induction in our study could be interpreted as resulting from novelty.

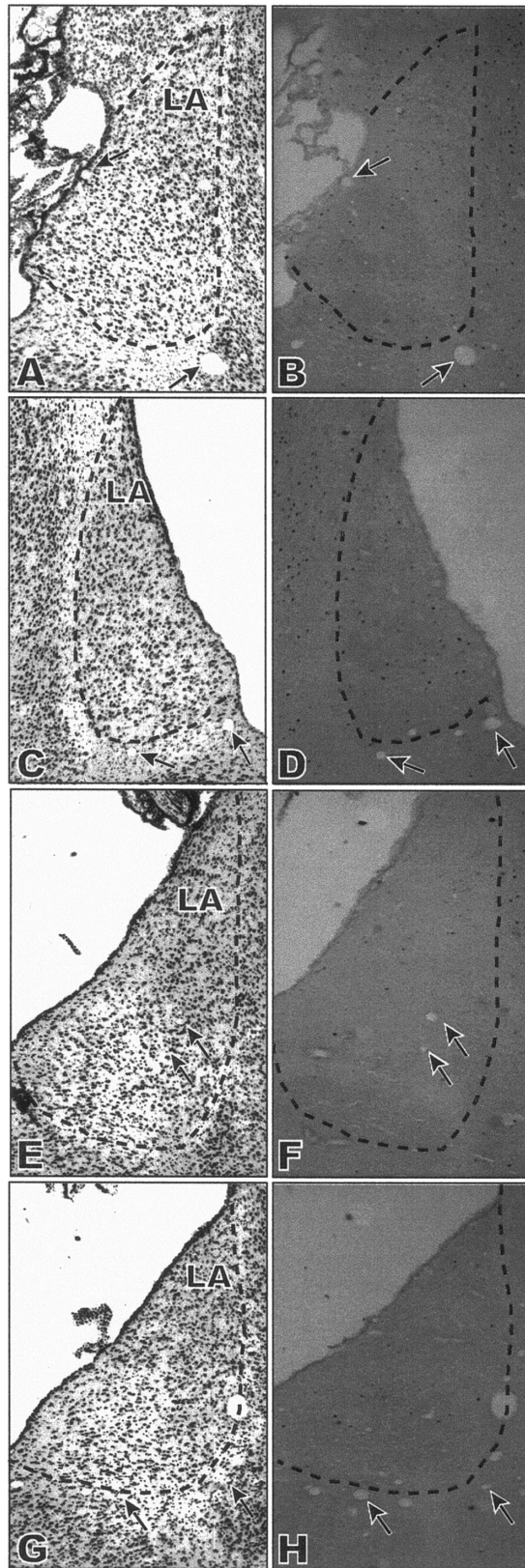


Figure 5. Brightfield photomicrographs of Nissl-stained (left) and Fos-stained (right) brain tissue in and around the lateral amygdalar

The present study is the first to show conditioned, stress-induced, lateralized expression of immediate early genes in the amygdala. We show that increased Fos levels in the right amygdala (CEAl and BLAA), as compared with the left amygdala, are associated with the contextual CS, consistent with a recent finding that the right amygdala has greater involvement in contextual conditioned fear than the left amygdala (Baker & Kim, 2004).

Our findings are also consistent with previous studies indicating that the right side of the amygdala is more involved in stress or emotionally related processes than the left (e.g., Adamec & Morgan, 1994; Andersen & Teicher, 1999). Of particular relevance here is the study of Coleman-Meschers and McGaugh (1995); they found that lidocaine inactivation of the right but not the left amygdala markedly impaired retention of a one-trial inhibitory avoidance task (male rats). In other studies, hemispheric asymmetries have been reported for response to fear, stress, and emotion (Carlson, Fitzgerald, Keller, & Glick, 1991, 1993; Carlson, Vicker, Keller, & Glick, 1996; Davidson, 1992; Denenberg, 1981; LaBar & LeDoux, 1996; Sullivan & Gratton, 1998, 1999).

Recent human brain imaging studies also report differential activation of the left and right amygdala by fearful emotional stimuli (e.g., Cahill et al., 1996; Morris, Frith, Perrett, Rowland, Young, Calder, & Dolan, 1996; Morris, Ohman, & Dolan, 1998). Cahill et al. (2001) reported a striking sex difference in the lateralization of amygdalar activation when viewing new, emotionally provocative films: Males showed enhanced activity in the right amygdala and females in the left. Canli, Desmond, Zhao, and Gabrieli (2002) found similar results when scanning during retention of emotional films: Men activated more structures in a network that included the right amygdala, whereas women activated a network including the left amygdala.

It is interesting to note that the two amygdalar cell groups that show lateralized Fos induction, the CEAl and BLAA, are unique in their connectional outputs. The CEAl has very restricted projections, with its major output to the fear conditioning circuit via projections to the CEAm (Petrovich & Swanson, 1997). The BLAA, on the other hand, has few if any direct projections to the CEA but instead sends heavy projections to the dorsal striatum and prefrontal cortex (Kita & Kitai, 1990; Swanson & Petrovich, 1998). Thus, greater involvement of the right CEAl and BLAA in conditioned-fear processing suggests differential influences of these structures on their output systems in the right hemisphere because the projections from the CEAl and BLAA are mainly ipsilateral.

In conclusion, our findings provide further evidence for the involvement of amygdalar cell groups in the retrieval and expression of contextual conditioned fear. We also provide evidence suggesting greater involvement of the right as compared with the left amygdala in processing fearful information. The detailed map of specific amygdalar regions that show Fos induction by conditioned stress in the present study may help guide future behavioral and physiological experiments. A better understanding of func-

nucleus (LA). Right side of the brain (A, B) and left side of the brain (C, D) transverse sections from Trained Group B. Right side of the brain transverse sections from control group (E, F) and shock-control group (G, H). Arrows point to corresponding blood vessels on both photomicrographs.

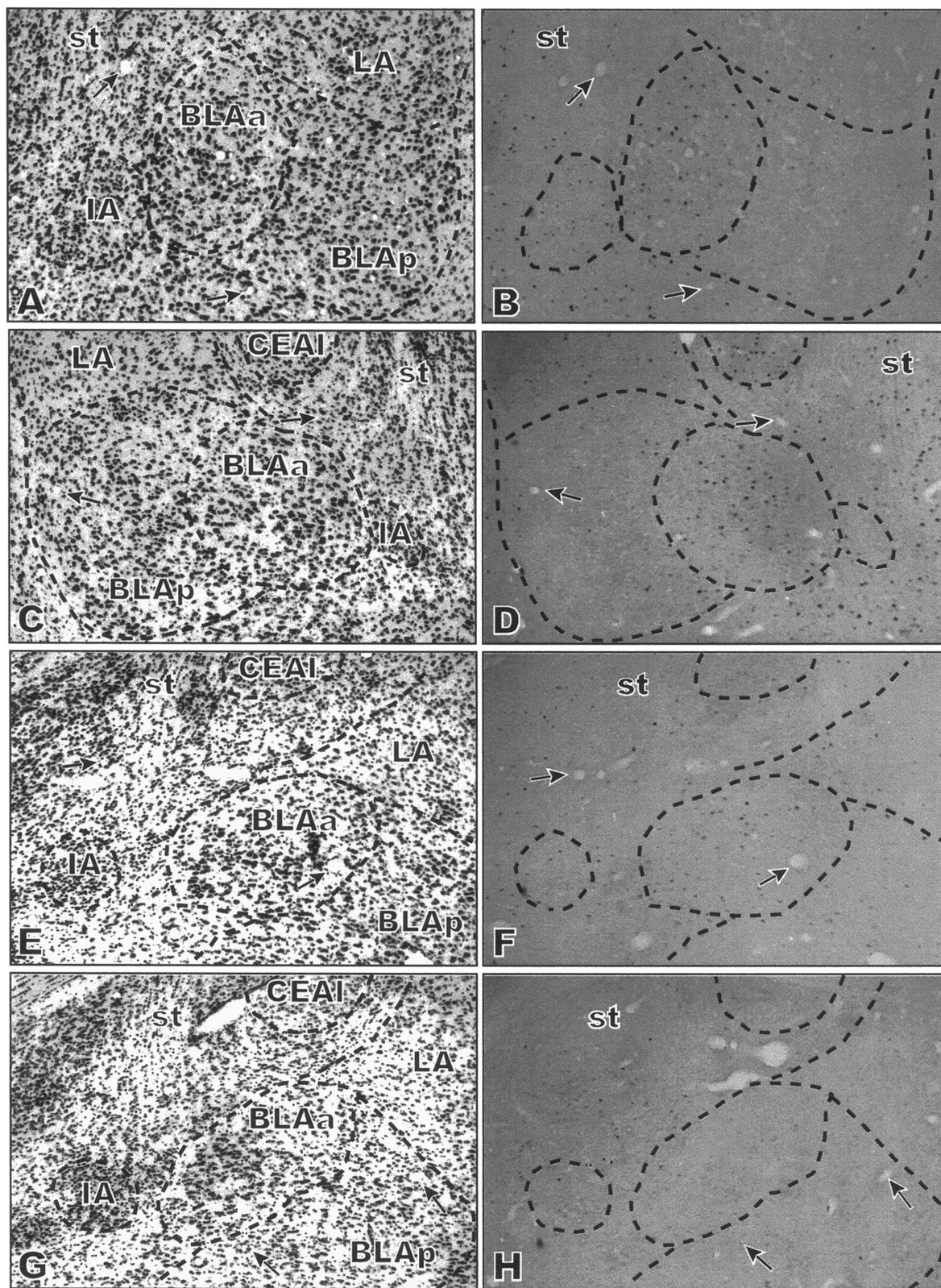


Figure 6. Brightfield photomicrographs of Nissl-stained (left) and Fos-stained (right) tissue in and around the basolateral amygdalar nucleus (BLA). Right side of the brain (A, B) and left side of the brain (C, D) transverse sections from Trained Group B. Right side of the brain transverse sections from control group (E, F) and shock-control group (G, H). Arrows point to corresponding blood vessels in both photomicrographs. BLAa = BLA, anterior part; BLAp = BLA, posterior part; CEAI = lateral part of the central amygdalar nucleus; LA = lateral amygdalar nuclei; st = stria terminalis; IA = intercalated amygdalar nuclei.

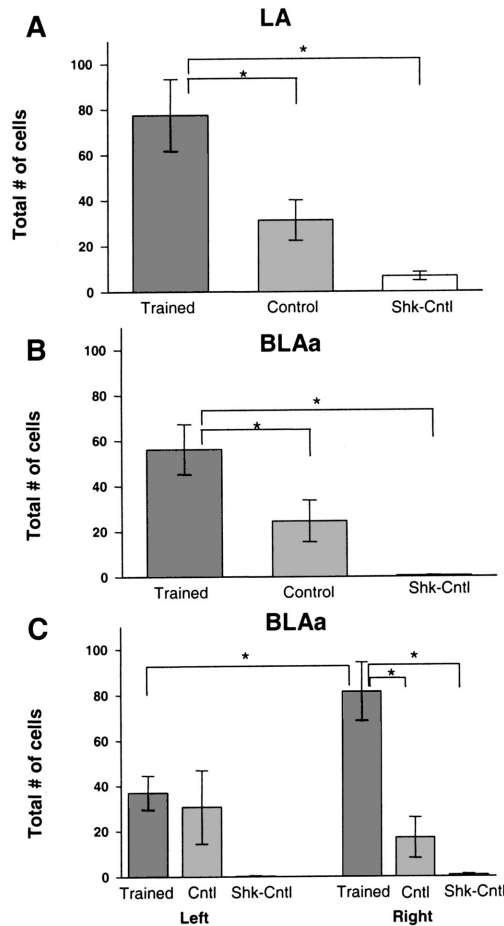


Figure 7. A: Number of Fos-immunoreactive neurons in the lateral amygdalar nucleus (LA) of the trained, control, and shock-control groups. B: Number of Fos-immunoreactive neurons within the anterior basolateral amygdalar nucleus (BLAa) in the trained, control, and shock-control groups. C: Number of Fos-immunoreactive neurons within the BLAa in the three groups on each side of the brain. Shk-cntl = shock-control. * $p < .01$.

tional lateralization within brain circuitry that processes learned fear could also help illuminate the pathologies associated with fear processing that include anxiety, depression, and phobias.

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