Contact with infants modulates anxiety-generated *c-fos* activity in the brains of postpartum rats

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Abstract

The postpartum period is associated with many behavioral changes, including a reduction in anxiety, which is thought to be necessary for mothers' ability to appropriately care for infants. In laboratory rats, this reduction in anxiety requires recent contact with pups, but areas of the brain where infant contact influences neural activity to reduce anxiety are mostly unknown. We examined *c-fos* expression in lactating rats whose pups were removed for 4 hours to increase mothers' anxiety, or not removed to maintain low anxiety in mothers, followed by exposure to the anxiogenic stimuli of either brief handling or handling followed by exposure to an elevated plus maze. Control animals had their litters removed or not, but no further stimulation. A large number of neural sites traditionally implicated in regulating anxiety in male rats were examined, and similar to what is found in male rats, most showed increased Fos expression after handling and/or elevated plus-maze exposure. Litter presence before testing affected Fos expression due to handling or elevated plus-maze exposure only in the ventral bed nucleus of the stria terminalis, dorsal and ventral preoptic area, ventromedial hypothalamus, lateral habenula, and supramammillary nucleus. Contrary to expectations, prior litter presence was associated with more Fos expression in most of these sites after handling and/or elevated plus maze stimulation, and only after such stimulation. These sites may be of particular importance for how sensory inputs from infants modulate anxiety and other mood states during the postpartum period.

Keywords

anxiety; elevated plus maze; immediate-early gene; lactation; postpartum; stress; suckling

1. Introduction

The postpartum period is a physically and emotionally demanding time of the reproductive cycle, and requires the coordination of numerous behavioral and physiological changes that maintain maternal homeostasis and promote offspring development. Reduced anxiety is one component of these changes, and occurs in both postpartum women and laboratory rats [for review see 43]. This reduction is critical for normal mother-infant interactions and infant development, because human or rodent mothers with high anxiety display altered or even completely aberrant maternal care [1,3,5,6,14,46–48,53,57,61,87–89,95].

The postpartum reduction in anxiety requires physical contact with offspring. In lactating women and rats, either suckling or non-suckling contact with infants can produce an anxiolytic effect [19,33,42]. Contact with infants must be recent, at least in rats, because anxiety-related

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behaviors in dams reverts back to the high levels found in diestrous virgin females if the litter is absent for even a few hours [42,59].

Areas of the brain affected by recent physical contact with infants to reduce anxiety in their mothers have not been explored in detail. Previous studies using peripheral or intracerebroventricular injections of receptor antagonists implicate numerous neurochemical systems, including GABA, norepinephrine, prolactin, and oxytocin [22,31,32,62,83,86], but do not address the specific sites of action. Lesion studies have been few, but reveal that the paraventricular hypothalamus is not involved in dams' exploration of a novel environment or freezing in response to a startling noise [64], and that destruction of the ventrocaudal periaqueductal gray further decreases dams' already low anxiety [45].

To explore where in the brain recent contact with pups might alter neural function in response to an anxiogenic experience, we examined *c-fos* expression in the brains of postpartum rats with low or high anxiety after they were subjected to brief handling or handling followed by exposure to an elevated plus maze. Low- and high-anxiety states were generated by permitting or preventing dams' recent contact with pups, respectively. The literature on the neural regulation of anxiety in females at any stage of the reproductive cycle is relatively small. Therefore, to help determine sites to be analyzed, we turned to the large literature on the neural control of anxiety in males, which implicates sites including the midline cortex, numerous areas of the hypothalamus, bed nucleus of the stria terminalis, septum, hippocampus, amygdala, and periaqueductal gray [9,10,52]. Parsimony suggests that the fundamental neural networks mediating anxiety-related behaviors are similar between the sexes, but that males and females are susceptible to different endocrine and sensory influences, including those resulting from physical contact with infants.

2. Materials and Methods

2.1 Subjects

Subjects were 55 Long-Evans female rats born and raised in our colony, but descended from rats purchased from Harlan Laboratories (Indianapolis, IN). After weaning at 21 days old, subjects were housed in clear polypropylene cages ($48 \times 28 \times 16$ cm) with woods shavings for bedding in groups of 2–3 littermates per cage. Beginning at 70 days old, females' estrous cycles were monitored daily with a vaginal impedance meter (Fine Science Tools, Foster City, CA), and females in proestrus placed overnight with a Long-Evans male from our colony. Mating was confirmed the next morning by copulatory plugs underneath the males' cages. Mated females were removed from males' cages and rehoused with one or two other recently inseminated females until 4–5 days before the expected day of parturition. Females were then individually housed and remained so with their litters throughout the remainder of the experiment. Litters were culled to four males and four females within 24 h after birth. The day of birth was assigned as day 0 postpartum. Food (Purina Rat Chow) and water were continuously available, lights were on a 12:12 light/dark cycle with onset at 0800 h daily, and the ambient temperature was ~ 22°C.

2.2 Experimental Design and Behavioral Testing

Dam and litter manipulation occurred between 0900–1000 hr on day 7 or 8 postpartum. To minimize disturbance before testing, anyone other than the experimenter was prohibited from entering the colony room on the day of testing. We used a between-subjects, 2-factor design with litter presence before testing as one factor (litter removed or not removed) and subsequent anxiogenic stimulation as the other factor (no stimulation, handling, or handling followed by exposure to an elevated plus maze). This resulted in six groups with final samples sizes of 8 animals in each group; an additional 7 dams fell off the elevated plus-maze and were

immediately removed from the study. Dams whose pups were not removed had the experimenter's hand placed in their home cage, and the pups picked up and immediately replaced, to control for perturbation caused by longer-term litter removal in females from the other condition. Four hours after litters were removed or not removed, experimental dams received one of two anxiogenic experiences - brief handling or handling followed by a 10-minute exposure to an elevated plus maze (see immediately below).

Handled subjects were carefully carried across the hallway from our colony room to a 10×10 ft testing room illuminated by a single 100-W bulb. Subjects were removed from their cage and very briefly suspended by their tail over an elevated plus maze. Subjects were immediately returned to their home cage, which contained the pups or not, and the cage then returned to the colony room.

For groups receiving exposure to an elevated plus maze, dams were carried in their home cage to the same testing room described above. The elevated plus maze was elevated 50 cm from the floor, and made of black plastic with four arms emerging from a 10×10 cm center square [68]. Arms measured 50×10 cm, two of which were open, while the other two had 40-cm-high walls. Illumination was ~28 lux on the open arms and ~2 lux at the end of enclosed arms. Each subject was removed from their home cage and placed in the central square of the maze facing an open arm and released. The home cage was then moved to an adjacent room. A Panasonic low-light-sensitive video camera relayed images of the plus maze from a mirror suspended above the maze to a Panasonic videocassette recorder and monitor in an adjacent room. An experimenter simultaneously recorded subjects' behavior with a computerized data-acquisition system that allowed recording the time spent in the open arms and closed arms, and the frequency of entries into each arm. An entry was recorded when the female placed her head and both front paws into an arm. Time spent in none of the arms was scored as time spent in the center square. After testing, dams were gently removed from the maze, and returned to their home cage (which contained the pups or not), which was carried back to the colony. The elevated plus maze was cleaned with 70% ethanol and dried between subjects.

Groups of control dams had their pups removed in the morning, or not removed, but these dams were not handled or exposed to the plus maze. They instead remained in the colony room, unmanipulated in their home cages with or without their pups until sacrifice.

2.3 Immunocytochemistry

One hour after experimental dams were handled or exposed to the elevated plus maze, or 5 hours after the morning litter manipulation for the unstimulated controls, subjects were overdosed with sodium pentobarbital and perfused through the heart with 150 ml saline followed by 150 ml 4% paraformaldehyde in 0.1M sodium phosphate buffered saline (PBS, pH 7.4). This time point was based on the maximal Fos protein expression in the rat brain following exposure to the elevated plus maze [17]. Brains were removed and postfixed overnight in 4% paraformaldehyde/PBS. On the following day, brains were placed in a 20% sucrose/PBS solution until sectioning. Each brain was cut into 35 μm sections and stored in a sucrose-based cryoprotectant at −20°C until immunocytochemical processing.

Every third section through the brain was immunocytochemically processed similar to that previously described [39]. Free floating sections were rinsed between steps, with the exception of after blocking with normal goat serum, three times in Trisma-buffered saline (TBS; pH = 7.6). Sections were first incubated in a 0.1% sodium borohydride/TBS solution for 15 min, 1% H_2O_2/TBS for 10 min, 20% normal goat serum with 0.3% Triton X-100 in TBS for 45 minutes, and 2% goat serum with 0.3% Triton X-100/TBS solution containing rabbit polyclonal anti-*c-fos* primary antibody (1:10,000; Santa Cruz Biotechnology, T-4037) at 4°C for 48 hrs. Tissue was then incubated for one hour in biotinylated anti-rabbit secondary antibody (1:500; Vector

Laboratories, Burlingame, CA) in 2% goat serum, a solution of avidin-biotin complex (Vectastain Elite; Vector Laboratories) for 60 min, and then a solution of 3,3′-diaminobenzidine (DAB), nickel ammonium sulfate, H_2O_2, in TBS. Sections were rinsed with TBS, mounted onto microscope slides, lightly counterstained with Neutral Red, dehydrated, and coverslipped.

2.4 Microscopic Analysis

Slides were randomized and coded so that experimenters were blind to group designation. Fos-IR cells were quantified at 200X magnification with the aid of a reticle placed on one ocular lens on a Nikon E400 microscope. Brain sites analyzed are shown in Figure 1, and a few additional areas were examined but are not reported because there was no or very low Fos immunoreactivity in any of the groups (*i.e.,* CA3, MHb, and MM). For each site, Fos-IR cells were counted bilaterally within a standardized area from one section for each subject, and sections were chosen based on their correspondence to the appropriate plate from Swanson's atlas of the rat brain [81]. Cells with any level of immunoreactivity were quantified by a single observer (C.D.S.).

2.5 Data analyses

Arm entries lasting <1 second were considered keystroke mistakes and removed from data analyses. The brain of one handled dam that remained with her pups was not analyzable, and she was removed from the study. One dam that had her pups removed before exposure to the elevated plus maze was found to be an outlier in the number of Fos-IR cells in the VMH (Dixon's outlier test, $p < 0.05$), and was removed from the analysis of that site. Behavior in the elevated plus maze was analyzed with independent *t*-tests. The number of Fos-IR cells in each neural site was analyzed by a 2×3 ANOVA using pup condition (pup present or pups absent before testing) and test condition (no stimulation, handling, or handling followed by exposure to an elevated plus maze) as factors. Statistical significance for main effects and interactions was indicated by $p \leq 0.05$. Main effects were followed by Bonferroni-corrected post-hoc analysis with $p \leq 0.016$ indicating statistically significant differences between groups. Pearson's *r* was used to examine correlations between Fos expression and behavior of the two groups of dams exposed to the elevated plus-maze.

3. Results

3.1 Elevated plus-maze behavior

Similar to our previous results [42], removal of the pups four hours before testing increased dams' anxiety-related behaviors in the elevated plus maze compared to dams allowed physical contact with pups until testing. Dams with their pups removed spent a significantly lower percentage of time in the open arms of the elevated plus maze ($t_{14} = 2.36$, $p \leq 0.05$; Figure 2a), and tended to have a lower percentage of entries made into open arms ($t_{14} = 1.84$, $p = 0.087$; Figure 2b). The raw duration of time spent in the open arms was also reduced by litter removal (89 ± 20 vs. 33 ± 11 sec; $t_{14} = 2.41$, $p \leq 0.04$), and the raw number of entries into open arms tended to be reduced (9 ± 2 vs. 5 ± 1; $t_{14} = 2.11$, $p = 0.054$). In contrast, the duration of time spent in the closed arms was somewhat higher for dams whose pups had been removed (445 ± 22 vs. 493 ± 18 sec; $t_{14} = 1.71$, $p = 0.10$). Removal of the litter did not significantly affect dams' total time spent in the arms of the maze (open plus closed; 526 ± 9 vs. 534 ± 6 sec; $t_{14} = 0.77$, $p \geq 0.45$), the number of entries into closed arms (11 ± 2 vs. 14 ± 1; $t_{14} = 1.43$, $p \geq 0.17$), or the total number of arm entries - although this tended to be lower for dams who had their pups removed (23 ± 2 vs. 16 ± 3; $t_{14} = 1.94$, $p = 0.07$).

3.2 Neural Fos expression - Main effects of anxiogenic stimulus

In 30 of the 31 sites analyzed, handling alone or handling followed by exposure to the elevated plus maze increased the number of cells containing Fos immunoreactivity compared to the lower number found in Unstimulated controls. Numerous patterns of main effects for stimulus were found (see Table 1 for data and site abbreviations): 1) A pattern where Unstimulated < Handled < Plus Maze was found in the CG, DG, NA shell, and MeApd. 2) A pattern where Unstimulated < Handled = Plus Maze was found in the LSvl, MS, BSTd and BSTv, mPOAd and mPOAv, NA core, ANH, DMH, PVNp, CeAm, LHb, PVT, PMd, SUM, rPAGd, and cPAGv. 3) A pattern of expression where Unstimulated < Plus Maze, but neither group differed from the intermediate levels found for the Handled group was found in the VMH, LH, PVNm, CA1, BLA, CeAl, MeApv, and cPAGd, and finally, 4) a pattern where Unstimulated = Handled < Plus Maze was found in the PMv. No differences between groups were found in the DR.

3.3 Neural Fos expression - Main effect of previous litter presence

Two sites showed significant main effects of previous litter presence on Fos expression. In the BSTv, when collapsed across stimulus type, dams that had their pups before testing and/or sacrifice had greater Fos expression ($F(1,41) = 10.27$, $p = .003$; Figure 3). Conversely, in the adjacent mPOAd, dams had fewer Fos-IR cells if their pups were present before testing and/or sacrifice ($F(1,41) = 8.21$, $p = .007$; Figure 3).

3.4 Neural Fos expression - Interactions between anxiogenic stimulus and previous litter presence

Statistically significant stimulus by litter presence interactions were found in the mPOAv (F $(2,41) = 4.82$, $p = .013$), VMH ($F(2,41) = 6.041$, $p = 0.005$), LHb ($F(2,41) = 4.93$, $p = .012$) and SUM ($F(2,41) = 3.40$, $p = 0.043$; Figure 4).

3.5 Correlations between Fos expression and elevated plus-maze behavior

Significant positive correlations between the number of Fos-IR cells and the percentage of time spent in the open arms of the elevated plus maze were found in the LHb ($r = 0.55$, $p \leq 0.03$) and cPAGd ($r = 0.56$, $p \leq 0.03$; Figure 5). These positive correlations were found only when both groups of dams exposed to the elevated plus maze (*i.e.,* dams with pups before testing and dams without pups before testing) were included in the analysis, but not when each group was analyzed separately.

4. Discussion

Numerous experiments have compared neural immediate-early gene activity in male rats differing in their responsiveness to anxiogenic stimuli, in order to provide insight into how the brain generates low- and high-anxiety states [36,37,70,71]. Using a similar strategy, our experiment compared Fos expression between postpartum rats showing low anxiety-related behavior due to the presence of pups prior to an anxiogenic experience, and dams showing higher anxiety-related behavior due to a lack of recent contact with pups. We identified a handful of neural sites that differed between these groups, and these sites may critical places of interface between the neural pathways processing sensory cues from infants and those modulating anxiety-related behaviors.

4.1 Fos expression in response to anxiogenic stimuli

The moderately anxiogenic and stressful experiences of handling, or handling followed by exposure to an elevated plus maze, significantly increased *c-fos* activity in most of the neural sites we analyzed. We consider these experiences moderately anxiogenic and stressful because they elicit lower hypothalamic-pituitary-adrenal (HPA) response compared to more severe

stimuli such as physical restraint, hypoxia, or forced swimming [40,54,60,62,93]. Our study is one of the few to examine immediate early-gene activity in the lactating rat brain after exposure to anxiogenic or stressful stimuli [12,72,94], and it is notable that almost all sites showing increased Fos expression in our mother rats have also been reported to show increased immediate early-gene expression when male rats are exposed to anxiogenic stimuli or anxiety-modulating drugs [13,15,17,18,25,30,49,51,69,71,73,74,76,77,82]. The neural networks underlying fundamental responses to anxiogenic stimuli, therefore, are probably quite similar in male and female rats. In females, though, activity of these networks can be uniquely modified by hormones and other neurochemicals fluctuating across the reproductive cycle [58,59,84, 85]. Furthermore, these fluctuations increase maternal motivation and behavior [44,63], which compels mothers to interact with their infants, thereby providing an additional factor that can lower mothers' anxiety.

4.2 Fos expression in response to anxiogenic stimuli is modulated by recent infant contact

If mother rats do not have recent contact with their infants, the percentage of time they spend in the open arms of an elevated plus maze is significantly reduced [42,59], and we replicate that finding here. We have recently demonstrated that the percentage of time spent in the open arms of an elevated plus maze is an appropriate reflection of anxiety in lactating rats, because it is reduced by anxiogenic drugs [67]. Interestingly, the percentage of open arm entries was not significantly affected by litter removal, although it approached statistical significance. Similarly, a previous experiment within an entire series of experiments from our lab found that the time spent in open arms significantly differed between lactating and diestrus virgin females, but that the frequency of entries into open arms did not [42]. Other experiments on lactating rats have also reported that open arm time and frequency of entries into open arms do not always have the same sensitivity to an experimental manipulation [62,83]. In fact, the classic studies of male rat behavior in an elevated plus maze by Pellow and colleagues [68] demonstrated that the time spent in open arms is more sensitive to anxiety-modulating drugs than is the frequency of entries into open arms, indicating that time spent in opens arms is a more reliable measure of anxiety-related behavior in this paradigm.

Our experiment is the first to address where in the brain recent sensory cues from pups affect neural modulation in response to an anxiogenic experience, and possibly influence mothers' anxiety-related behaviors. This was accomplished by examining Fos expression after manipulating the presence of the litter, and thereby mothers' anxiety, before exposing dams to anxiogenic stimuli. This procedure allowed us to compare high and low-anxiety female rats otherwise identical in their reproductive experience and previous hormonal and neurochemical milieu, an advantage that would be lost if anxiety and Fos expression were compared between lactating and virgin females. Studies examining Fos expression after a stressor (restraint, urethane, or endotoxin) find that mothers have fewer Fos-containing cells compared to virgins in numerous sites, including the PVN, supraoptic nucleus, medial amygdala, ventrolateral septum, and cingulate cortex [12,72,94]. We found that Fos expression in none of these sites differed between low-anxiety and high-anxiety dams. This could be due to differences in the specific stressful and anxiogenic stimulus used, but could also imply that reproductive state and recent contact with infants have unique influences on anxiety- and stress-induced Fos expression in some neural sites.

Although removal of pups increases dams' anxiety-related behaviors, Fos expression in most neural sites we examined was not affected by whether or not the pups were present before testing. Two neural sites showed significant main effects of prior litter presence on Fos expression (mPOAd and BSTv), and four had a significant litter presence by stimulus interaction (mPOAv, LHb, VMH, SUM). This is consistent with the finding that low- or high-anxiety males differ in Fos expression after presentation of anxiogenic stimuli in some, but not

most, neural sites implicated in anxiety [36,37,70,71]. The sites differing between low- and high-anxiety males (CG, LSvl, PVN, AHN, LH, CA1, CA3, DG, PVT, and rPAGd) were not the same as those that differed between our low- and high-anxiety females. Thus, even if the fundamental neural networks responding to anxiogenic stimuli are the same in male and female rats, the sites specifically responsible for generating low-and high-anxiety responses *within* each sex may differ between males and females. Indeed, the six sites we found affected by litter presence seem uniquely suited to modulate anxiety in postpartum females. These sites are directly or indirectly connected [8,16,20,28,75], are responsive to sensory cues from pups and necessary for the display of maternal behaviors [63,92], as well as influence emotional responses in rodents [2,4,26,27,34,56,65,91]. They might be important sites of interface between the neural networks balancing dams' maternal and anxiety-related behaviors.

We believe there are two points of particular interest regarding these six sites. First, in five of them (mPOAd, mPOAv, LHb, SUM, VMH), and even in some sites that did not have significant main effects of or interactions with litter presence (*e.g.,* CG, BSTd, MS, LSvl, PVNp, NAc, LH), a comparison of the unstimulated controls reveals that simply removing the litter produced a 2–6-fold increase in Fos expression. This is not the result of a physiological stress response, because temporary removal of the litter actually decreases maternal glucocorticoid levels, not increases them [24,78,90]. One of the largest increases in Fos after removal of the litter was in the LHb. The LHb is necessary for the onset of maternal behavior in rats [20,50], and shows increased Fos expression after mothers and litters are reunited following a 2-day separation [45]. This increase occurs only if the litter is removed again before dams are sacrificed [45], but not if the litter and dam remain together until sacrifice [79]. Our data reveal that Fos expression also increases in the LHb after litter removal even following otherwise undisturbed mother-litter interactions. The relevance of increased immediately early-gene activity in the LHb or any other sites after dams are separated from their infants remains to be determined, but it could be related to short-term behavioral and physiological changes that normally occur when mothers leave their nest, including anxiety that increases when dams spend time away from their infants.

The second interesting finding was that the presence of pups had a generally permissive effect on dams' Fos expression in response to elevated plus-maze exposure, even though these dams displayed less-anxious behavior. We also found only positive correlations, two of which were statistically significant, between the number of Fos-IR cells in any site and the percentage of time spent in the open arms of the maze. The need to collapse across plus-maze groups to find the significant correlations is probably because we needed a sample large enough to provide sufficient variability, particularly on the behavioral variable, before a correlation of any strength could be revealed. This relationship between high Fos expression and low-anxiety behaviors was not completely unexpected, and quite a few neural sites (CG, PVT, LHb, CA1, CA3, and DG) show higher Fos expression after anxiogenic experiences in male rats that are less anxious compared to males that are more anxious [36,37,70,71].

It is valuable to note that, even though Fos expression in some sites increased in controls simply by removing the litter, it was the groups with their pups present before handling or plus-maze exposure that had the highest Fos expression in most areas. This permissive effect of infant presence on Fos expression was greatest in the BSTv, where dams with their litter present before exposure to the elevated plus maze had twice as many Fos-IR cells compared to dams who did not have their litter present before exposure to the maze Importantly, mothers with or without pups that remained unstimulated had a similarly low number of Fos-IR cells in the BSTv, so Fos expression was not due to the performance of maternal behaviors during the four hours before handling or exposure to the elevated plus maze. Anxiety-induced Fos expression in the BSTv, and the other sites where there was a permissive effect of prior litter presence, may occur selectively in GABAergic neurons. The BSTv is an important relay between

hypothalamic, amygdalar, and brainstem sites mediating anxiety and stress [91]. It is highly sensitive to norepinephrine [23], and most of its neurons are GABAergic [11,55,80]. Reduced noradrenergic tone when mother are in contact with infants [86] might disinhibit GABA cells in the BSTv [23], thereby suppressing downstream neural activity, and conveying a sense of "safety" that reduces anxiety-related behaviors [7]. Future research is required to determine the phenotype of Fos-immunoreactive cells in the BSTv, and elsewhere in the brain, after lactating rats are exposed to anxiogenic stimuli to help reveal the possibility of such mechanisms.

The exception to the facilitatory effect of pup presence on anxiety-induced Fos expression was the mPOAd, where previous litter presence had a generally suppressive effect. The majority of cells in the mPOAd are also GABAergic [55], but some of its projection neurons to neural areas modulating anxiety and stress responses - such as the posterior hypothalamus and PAG - are not [29,35,66]. The simplest hypothesis is that these excitatory projections need to be suppressed in low-anxiety mothers, which is reflected by low *c-fos* activity when mothers are faced with anxiogenic stimuli. Again, determining the phenotype of dams' Fos-immunoreactive cells will be necessary to support this possibility.

In conclusion, lactating rats subjected to an anxiogenic experience show increased Fos expression in many neural areas traditionally implicated in regulating anxiety and stress. Recent contact with pups influences this Fos expression in a handful of sites that could be important starting points to examine how infant contact produces an anxiolytic effect in their mothers. A variety of neurochemicals released in or by these sites - including GABA, norepinephrine, oxytocin, prolactin -might be involved in this process [38,43,58]. Understanding how infant contact modulates the variety of neurochemicals capable of modulating anxiety in postpartum rats will likely contribute to a better understanding of anxiety disorders in postpartum women, and how these disorders can be alleviated by physical contact with infants.

Acknowledgments

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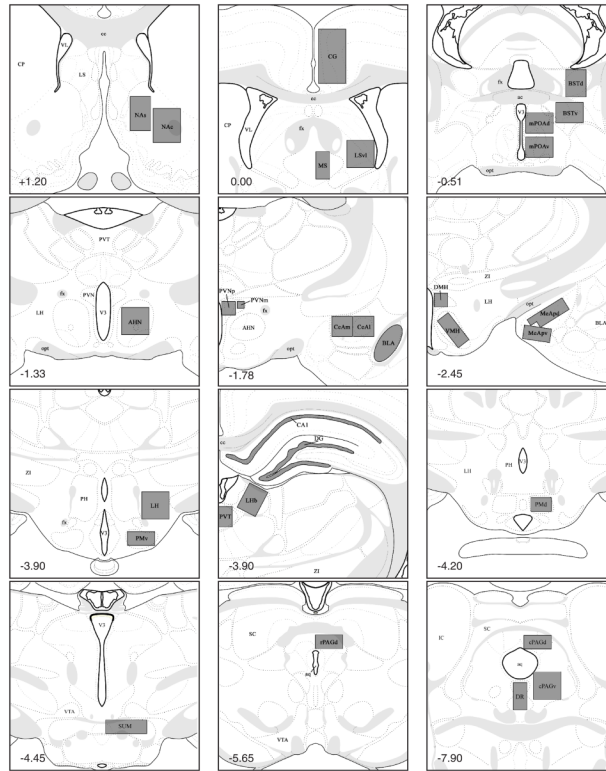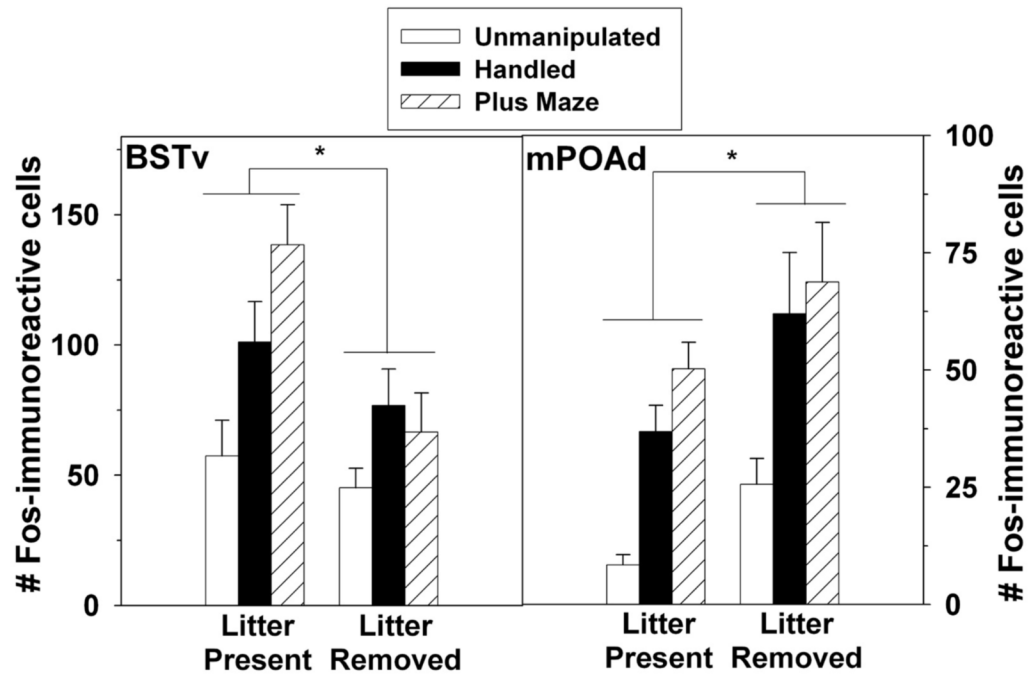
Figure 1.
Schematic representation of brain regions where Fos immunoreactive cells were quantified. Gray boxes indicate area of analysis, and numbers in bottom left of each panel indicate distance from bregma (in mm). All plates modified from Swanson (1998). Abbreviations: AHN - anterior hypothalamus; aq - cerebral aqueduct; BLAm - medial basolateral amygdala; BSTdm -dorsomedial bed nucleus of the stria teriminalis; BSTvm - ventromedial bed nucleus of the stria terminalis; CA1 - CA1 field of the hippocampus; cc - corpus callosum; CeAl - anterolateral central amygdala; CeAm - anteromedial central amygdala; CG - cingulate cortex; CP - caudate putamen; cPAGd - dorsocaudal periaqueductal gray; cPAGv - ventrocaudal periaqueductal gray; DG - dentate gyrus; DMH - dorsomedial hypothalamus; DR - dorsal raphe; fx - fornix; IC - inferior colliculus; LH - lateral hypothalamus; LHb - lateral habenula; LSvl - ventrolateral septum; MeApd - posterodorsal medial amygdala; MeApv - posteroventral medial amygdala; mPOAd - dorsal medial preoptic area; mPOAv - ventral medial preoptic area; MS - medial septum; NAc - nucleus accumbens core; NAs -nucleus accumbens shell; opt - optic tract; PMd - dorsal premammillary nucleus; PMv - ventral premammillary nucleus; PVNm - magnocellular paraventricular hypothalamus; PVNp -parvocellular paraventricular hypothalamus; PVT - paraventricular thalamic nucleus; rPAGd -dorsorostral periaqueductal gray; SC - superior colliculus; SUM - supramammillary nucleus; V3 -third ventricle; VL - lateral ventricle; VMH - ventromedial hypothalamus; VTA - ventral tegmental area; ZI - zona incerta.
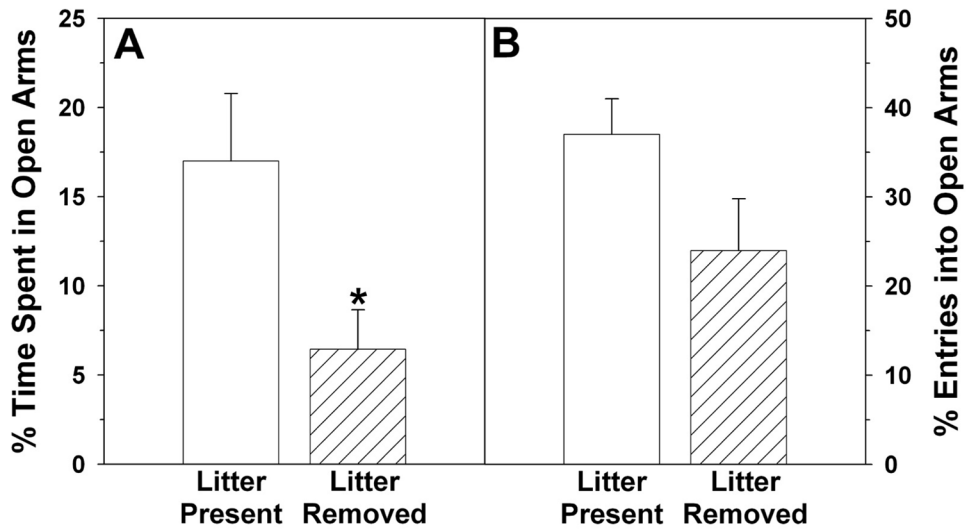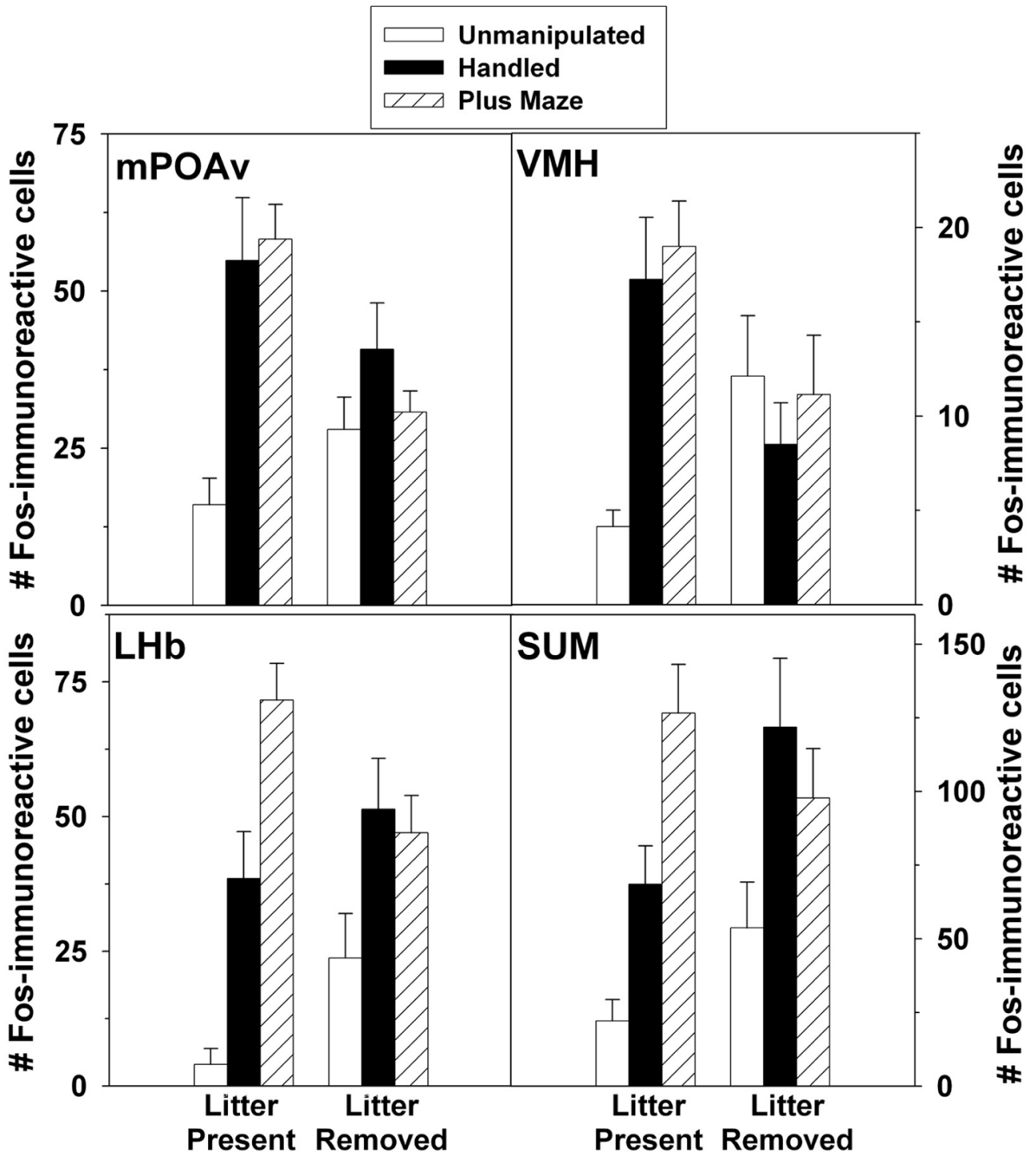
Figure 2.
Percentage of *A*) time spent and *B*) entries made in the open arms of an elevated plus maze by lactating rats that had their pups present for the four hours before testing (Litter Present) or had their pups removed (Litter Removed).
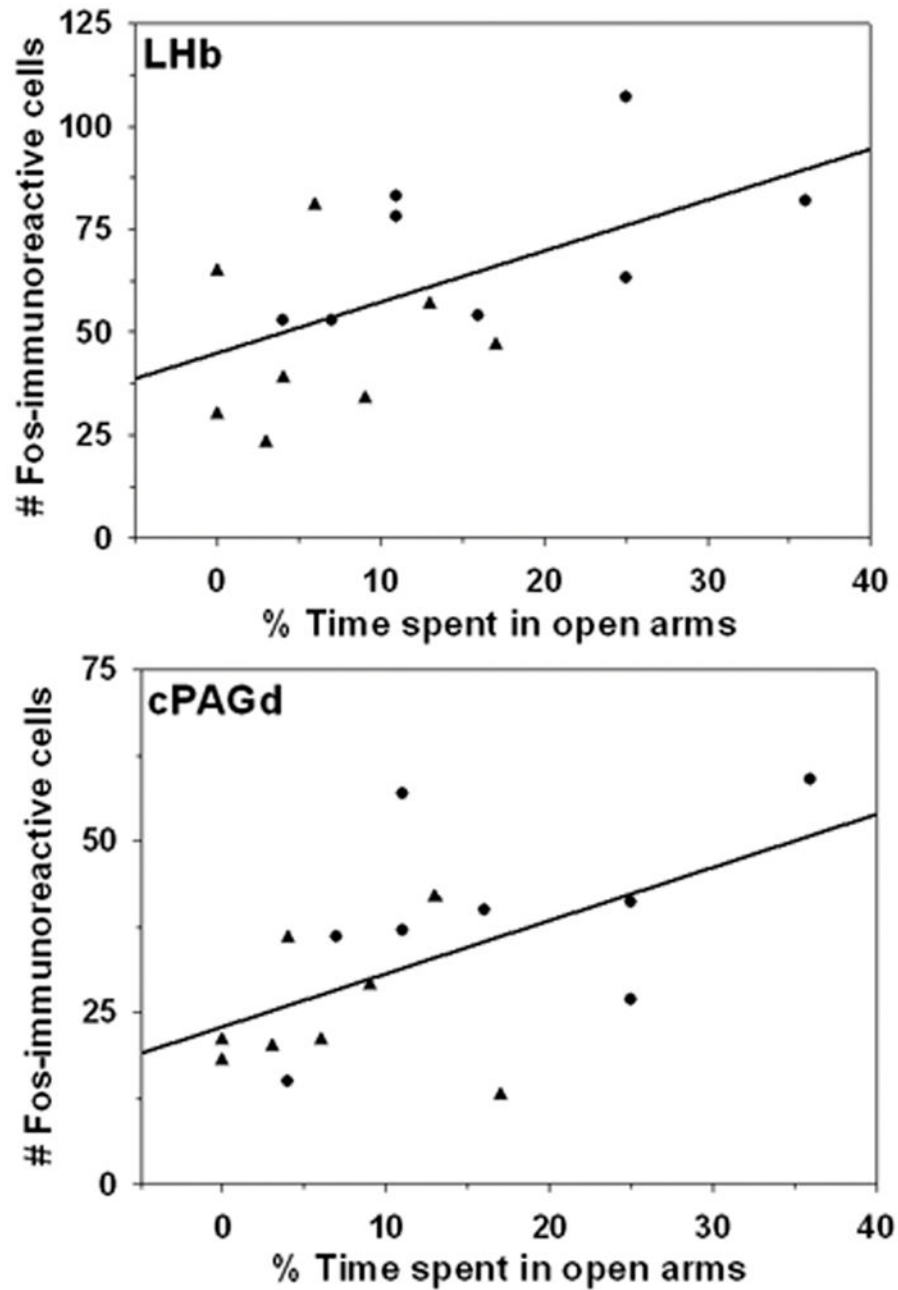
Figure 3.
Number (Mean ± SEM) number of Fos-immunoreactive cells in the BSTv and mPOAd of postpartum rats exposed to no stimulation, handling, or an elevated plus maze. Subjects had their pups present for the four hours before testing (Litter Present) or had the pups removed (Litter Removed). Note that X axes differ across panels.

Figure 4.
Number (Mean ± SEM) number of Fos-immunoreactive cells in the mPOAv, VMH, LHb, and SUM of postpartum rats exposed to no stimulation, handling, or an elevated plus maze. Subjects had their pups present for the four hours before testing (Litter Present) or had the pups removed (Litter Removed). Note that X-axes differ across panels.

Figure 5.
Correlations between the number of Fos-immunoreactive cells in the LHb (top) and cPAGd (bottom) and the percentage of time dams spent in open arms of an elevated plus maze. Circles represent dams with pups before testing, and triangles indicate dams whose pups were removed before testing.

Table 1

Number (Mean ± SEM) of Fos-immunoreactive cells quantified bilaterally from one section per site in the brains of lactating rats that received no stimulation (Unstimulated), brief handling (Handled), or exposure to an elevated plus maze (Plus Maze) after their pups were removed or not removed four hours before testing. Sites are grouped according to pattern of significant differences between groups when collapsed across pup condition, with Bonferroni-corrected post-hoc $p < 0.016$ indicating statistical significance.

	Unstimulated		Handled		Plus Maze		$F_{2,41}$	p
	With pups	Pups removed	With pups	Pups removed	With pups	Pups removed		
Unstimulated < Handled < Plus Maze								
CG	7 ± 3	32 ± 14	73 ± 21	94 ± 15	207 ± 24	173 ± 34	35.95	0.0001
DG	11 ± 3	11 ± 3	25 ± 8	30 ± 6	49 ± 9	40 ± 7	13.99	0.0001
NA$_s$	31 ± 6	56 ± 12	83 ± 15	95 ± 14	131 ± 12	116 ± 17	17.69	0.0001
MeA$_{pd}$	12 ± 6	19 ± 2	37 ± 8	40 ± 8	95 ± 7	84 ± 13	43.47	0.0001
Unstimulated < Handled = Plus Maze								
LS$_{vl}$	12 ± 7	50 ± 20	149 ± 23	156 ± 25	184 ± 23	177 ± 33	22.30	0.0001
MS	4 ± 1	20 ± 8	41 ± 6	53 ± 10	66 ± 6	48 ± 9	20.31	0.0001
BST$_d$	11 ± 3	28 ± 9	35 ± 8	55 ± 10	61 ± 6	65 ± 13	11.94	0.0001
NA$_c$	8 ± 2	20 ± 7	42 ± 9	46 ± 7	64 ± 10	56 ± 12	15.01	0.0001
AHN	35 ± 7	60 ± 8	85 ± 15	93 ± 15	128 ± 14	101 ± 19	11.82	0.0001
DMH	23 ± 5	25 ± 11	54 ± 8	51 ± 11	87 ± 14	46 ± 13	7.80	0.0013
PVN$_p$	3 ± 1	11 ± 3	23 ± 6	32 ± 7	42 ± 6	45 ± 11	14.90	0.0001
CeA$_m$	4 ± 2	4 ± 1	15 ± 5	12 ± 4	17 ± 3	10 ± 3	5.85	0.0058
PVT	16 ± 3	29 ± 4	37 ± 8	46 ± 8	36 ± 5	44 ± 10	4.58	0.0160
PMd	6 ± 2	8 ± 2	25 ± 11	16 ± 6	27 ± 6	25 ± 6	5.00	0.0114
rPAG$_d$	6 ± 1	10 ± 3	17 ± 5	22 ± 5	30 ± 4	25 ± 6	10.03	0.0003
cPAG$_v$	11 ± 3	14 ± 5	26 ± 8	31 ± 8	32 ± 6	29 ± 6	4.36	0.0191
Unstimulated < Plus Maze*								
LH	4 ± 2	11 ± 4	19 ± 6	27 ± 7	39 ± 5	35 ± 10	10.58	0.0002
PVN$_m$	0 ± 0	1 ± 0	8 ± 3	3 ± 1	9 ± 4	6 ± 3	4.09	0.0241
CA1	0 ± 0	1 ± 0	4 ± 2	3 ± 2	7 ± 2	7 ± 3	4.70	0.0145
BLA	1 ± 1	4 ± 2	19 ± 10	16 ± 2	47 ± 10	22 ± 9	9.88	0.0003

	Unstimulated		Handled		Plus Maze		$F_{2,41}$	p
	With pups	Pups removed	With pups	Pups removed	With pups	Pups removed		
CeA$_l$	2 ± 1	6 ± 3	7 ± 2	16 ± 3	12 ± 2	12 ± 5	4.15	0.0229
MeA$_{pv}$	11 ± 2	16 ± 3	36 ± 5	26 ± 6	70 ± 6	74 ± 14	33.55	0.0001
cPAG$_d$	13 ± 3	16 ± 5	21 ± 6	26 ± 6	39 ± 5	25 ± 4	6.14	0.0047
Unstimulated = Handled < Plus Maze								
PM$_v$	4 ± 1	8 ± 3	16 ± 4	18 ± 5	39 ± 9	32 ± 12	9.11	0.0005
Unstimulated = Handled = Plus Maze								
DR	0 ± 0	5 ± 5	12 ± 6	11 ± 4	5 ± 1	6 ± 2	2.39	0.1041

Data from additional sites shown in Figures 3 and 4. Statistical information provided in right-hand columns is for the main effects of stimulus. Abbreviations as in Figure 1.

* = Neither group differs from the intermediate levels found in Handled dams.