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Maternal Behavior Stimulates *c-fos* Activity within Estrogen Receptor Alpha-Containing Neurons in Lactating Rats

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Key Words

c-fos · Immunocytochemistry · Lactation · Maternal behavior · Gonadal steroid receptors · Amygdala · Preoptic area · Bed nucleus of the stria terminalis · Habenula

Abstract

Estradiol and other hormones are thought to be critical for the onset, but not maintenance, of maternal behavior in rats. Maternal behavior is instead maintained postpartum by tactile stimulation that dams receive during interactions with pups, and many neural sites implicated in the control of maternal behavior show elevated *c-fos* activity in response to this stimulation. Many of these sites also contain neurons that express the alpha subtype of the estrogen receptor (ERα). Because of possible interactions between tactile stimulation from pups, *c-fos,* and ERα in the lactating rat brain, we determined if populations of cells that show increased *c-fos* activity after maternal behavior in lactating rats also contain ERα. Dams were separated from their pups for 48 h beginning on day 5 postpartum. On day 7 postpartum, experimental dams were reunited with pups and mother-litter interactions were observed for 60 min. Control dams received no pup stimulation. Subjects were sacrificed 60 min later and brain sections were double immunolabeled for the Fos and ERα proteins. As expected, the number of ERα-immunoreactive (ERα-ir) neurons did not differ between the two groups in the eight areas analyzed (lateral region of the lateral septum, posterodorsal medial amygdala, dorsal and ventral medial preoptic area, dorsal and ventral bed nucleus of the stria terminalis, lateral habenula, and ventrolateral caudal periaqueductal gray). Consistent with previous reports, maternal dams had 2- to 7-fold more Fos-immunoreactive (Fos-ir) neurons in these sites compared with nonstimulated controls. Maternal dams had significantly more Fos-ir neurons that also contained ERα-ir in all sites, with the greatest increases in the ventral medial preoptic area, lateral habenula, and ventral bed nucleus of the stria terminalis. Between ~25 and 45% of the Fos-ir cells in the sites examined also expressed ERα. Thus, a substantial number of neurons that are genomically activated during maternal behavior contain ERα, raising the possibility that the postpartum display of maternal behavior can be influenced by ERα activity.

Adult female rats are typically not maternal until undergoing the fluctuations in ovarian and pituitary hormones associated with gestation and parturition [1–3]. Ovarian hormones secreted during mid- to late pregnancy may be particularly important for the onset of nurturance [4], and a regimen of hormones mimicking their pattern of release induces maternal responding in virgin albino female rats [5]. Estradiol may be especially critical for this

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behavioral change in female rats and systemic administration of high doses of estradiol alone, but not progesterone, promotes maternal responses in virgin females [5]. There are many areas of the brain that may be influenced by estradiol for the display of maternal behavior, and many neural sites known to be important for the onset and/or postpartum display of maternal care in rats [6–14] contain the α subtype of the estrogen receptor (ERα) [15]. A role for estrogen receptor activity in the display of maternal behavior is supported by the fact that maternal responding can be facilitated in pregnancy-terminated female rats via direct administration of estradiol into at least one site that contains neurons expressing ERα [15] – the medial preoptic area (mPOA) [6]. Furthermore, the amount of occupied estrogen receptors in the mPOA is chronically elevated beginning at mid-pregnancy [16, 17], which may be important for the periparturitional onset of maternal behavior.

While ovarian and pituitary hormones are critical for the initiation of maternal behavior, they are not necessary for its postpartum maintenance [1–3]. Rather, postpartum maternal responsiveness is maintained by the very tactile cues from pups that elicit maternal behavior in the dam [3, 18, 19]. The neural mechanisms underlying the transition from the hormonally facilitated onset of maternal behavior to its nonhormonal maintenance throughout lactation are not known. However, it is likely that many brain areas that respond to hormones to facilitate initial maternal responsiveness to pup stimuli are also important for its hormone-independent display (e.g. mPOA) [2].

Many neural populations are stimulated during maternal behavior in lactating rats, and this activity can be visualized by immunocytochemical detection of the immediate early gene products Fos and FosB as a marker for neuronal modulation [13, 20–27]. In most cases, direct physical contact with pups and receipt of tactile stimulation from them is necessary for this increase in immediate early gene activity [13, 20–27]. Moreover, the genomic cascade initiated by activation of these immediate-early genes may be functionally significant for maternal behavior [28]. Little is known about the phenotype of neurons that show increased immediate-early gene activity during the performance of maternal behavior in lactating rats other than the fact that approximately half of the Fos-ir neurons in the mPOA, ventral bed nucleus of the stria terminalis (vBST), and ventrolateral caudal periaqueductal gray ($cPAG_{vl}$) of maternal rats are GABAergic [29]. It has been noted, however, that many sites that contain Fos- and FosB-expressing cells in maternally behaving rats also express ERα [24, 30]. This is true when maternal behavior is tested soon after parturition at a time when the dams' behavior has recently been under the influence of estradiol [20, 21] as well as many days later when the behavior is no longer hormone-dependent [13, 22–27]. The presence of ERα in sites with elevated Fos-ir suggests the possibility that many of these neurons co-express Fos and ERα.

Intraneuronal convergence of immediate-early gene activity and steroid hormone receptors has been demonstrated in the brains of sexually stimulated female rats [31, 32]. While steroid receptors have long been assumed to require their hormone ligand for activation, recent studies demonstrate activation of steroid receptors by neurotransmitters in the absence of hormone [ligand-independent activation; see 33]. Thus, it is conceivable that areas of the postpartum rat brain that are relevant for maternal behavior can be influenced by ERα activity by a similar process, even though circulating levels of estradiol are very low during lactation [34, 35]. We have tested this hypothesis by examining a possible overlap in the populations of neurons that express ERα and those that show elevated Fos expression after the display of postpartum maternal behavior in lactating rats.

Materials and Methods

Subjects

Subjects were 19 Sprague-Dawley female rats (Taconic, Germantown, N.Y., USA) purchased at 65–75 days of age and mated with sexually experienced Sprague-Dawley males from our colony one week after arrival. Females were housed in groups of 2–3 animals in wire hanging cages until 3–4 days prior to the expected day of parturition, after which they were individually housed in clear polypropylene cages (48 × 28 × 16 cm) with wood shaving for bedding. Dams were then placed in a small colony room containing pregnant females and lactating dams with their litters for the remainder of the experiment. Dams were completely undisturbed during the 48-hour separation from pups. Food and water were available ad libitum, lights were on between 08.00 and 16.00 h daily, and the ambient temperature was ~22 ± 1°C. Litters were culled to contain 8 pups (4 males, 4 females) within 24 h after parturition. During 48-hour mother-litter separations, litters were given to surrogate lactating dams from our colony of the same lactational stage as the biological mother.

Behavioral Testing

On the morning of day 5 postpartum (PP), dams had their litters removed and were rehoused in clean clear polypropylene pan cages with clean bedding. Forty-eight hours later, dams received either pup stimulation (n = 10) or no stimulation (n = 9). For dams receiving pup stimulation, litters were removed from surrogate lactating dams on the morning of day 7 PP between 09.00 and 10.30 h and incubated at nest temperature (~ 34°C) in a paper-lined glass bowl for 3 h prior to behavioral testing. After the 3-h incubation, pups were expressed of feces and urine and weighed. Subject's cage tops were

briefly removed and litters were scattered in the home cage diagonally opposite to where the dam was sitting. Mother-litter interactions were continuously observed for 60 min with the aid of a computerized data acquisition system that provided information on behavioral frequencies, latencies, and durations of the dams' active and inactive behaviors as described previously [13]. Pup stretch responses to milk receipt [36] were also recorded. After the behavioral observation, pups were immediately removed from the dam's cage and weighed. Nonstimulated dams had their cagetops briefly removed and replaced as if pups were being introduced, and again 60 min later as if pups were being removed. All subjects remained alone in their home cage for another 60 min before being anesthetized with an overdose of pentobarbital (Sigma, USA) and perfused.

Immunocytochemistry

Dams were perfused through the heart with 150 ml of 0.9% saline followed by 150 ml of 4% paraformaldehyde (Sigma, USA) dissolved in 0.1 *M* sodium phosphate buffered saline (PBS, pH 7.4). Brains were removed and postfixed overnight in 4% paraformaldehyde and submerged in 30% sucrose in 0.1 *M* PBS (pH 7.4) for at least 3 days before sectioning. Within 5 days after perfusion, entire brains were cut on a freezing microtome into 35-μm coronal sections which were stored in PBS-based cryoprotectant (pH 7.4) until immunocytochemical processing. Every fourth section through the brain was processed immunocytochemically for Fos-ir and ERα-ir. Free-floating sections were washed three times in 0.5 *M* Trisma-buffered saline (TBS, pH 7.6) for 5 min each rinse, incubated for 15 min in 0.5% sodium borohydride, washed three times in TBS, incubated for 45 min in 20% normal goat serum in 0.3% Triton X-100 in TBS, and incubated for 48 h at 4°C in a solution of 0.5 *M* TBS with 0.02% sodium azide, 0.1% gelatin and 0.02% Triton X-100 containing a rabbit polyclonal anti-*c-fos* antibody that recognizes residues 4–17 of the human Fos protein (Ab-5, 1:1,000; Oncogene Science, Manhassat, N.Y., USA) and a mouse monoclonal antibody for the ERα protein that recognizes the N-terminal of the human estrogen receptor and recognizes both occupied and unoccupied estrogen receptors (1D5, 1:500; DAKO, Glostrup, Denmark). Sections were again washed in TBS and incubated for 2 h in a mixture of two fluorescent secondary antibodies (Jackson ImmunoResearch, West Grove, Pa., USA): Fluorescein-conjugated goat antirabbit (1:50) for visualization of Fos and cyanine-conjugated goat antimouse (1:50) for visualization of ERα. Sections were rinsed in TBS followed by distilled water and mounted onto microscope slides. While sections were slightly wet, slides were coverslipped using Vectashield mounting medium (Vector Labs, Burlingame, Calif., USA), which preserves immunofluorescent staining. Three immunocytochemical runs were used, with brain sections from three subjects per group represented in each.

Controls for immunocytochemical specificity included sections incubated with no primary antiserum and the two secondary antisera together, no primary antiserum and each of the secondary antisersa individually, each primary antiserum alone with the two secondary antisera together, and each primary antiserum alone with each of the two secondary antisera alone. No cross-reactivity was observed between the two primary antisera (i.e. no cyanine immunoreactivity was observed if ERα primary antiserum was omitted and no fluorescein immunoreactivity was observed is Fos primary antiserum was omitted). Furthermore, no specific fluorescent staining was observed if primary antisera were omitted or if the primary antisera were run individually with the opposite secondary antiserum (i.e. ERα with fluorescein or Fos with cyanine).

Microscopic Analysis

Within 18 h after coverslipping, slides were randomized and coded for microscopic analysis. Eight neural sites that show large increases in Fos-ir neurons specifically after maternal behavior and direct physical contact with pups in lactating dams [13, 20–27] and populations of cells that express ERα [15] were then analyzed (fig. 1). These sites included (rostral to caudal): the lateral region of the lateral septum (LS_l) at the level approximately –0.00 mm from bregma and corresponding to atlas plate 17 from Swanson's [37] atlas of the rat brain, the dorsal and ventral medial preoptic areas ($mPOA_d$ and $mPOA_v$; –0.46 mm, plate 20), the dorsal bed nucleus of the stria terminalis primarily encompassing the region of the principal nucleus (BST_d), the ventral BST primarily encompassing its ventral nucleus (BST_v; both BST sites –0.51 mm, plate 21), the posterodorsal medial amygdala (MeA_{pd}; –2.45 mm, plate 28), lateral habenula (LHb; –3.90 mm, plate 32), and the ventrolateral caudal periaqueductal gray ($cPAG_{vl}$; –7.60, plate 44). The particular levels of analysis for the mPOA, BST_d, BST_v, LHb, and $cPAG_{vl}$ were chosen because they closely corresponded to the levels known to express the greatest number of Fos-ir nuclei after the display of maternal behavior in female rats [13, 23, 25, 26, 38]. There has not been a finely detailed analysis of Fos-ir at different rostrocaudal levels of the LS and MeA_{pd} after maternal behavior, so the levels of analysis chosen for these sites were similar to those used in previous reports of Fos expression after maternal behavior [24–26, 38].

In addition, three control sites were also examined bilaterally, but not quantified, for each subject in order to determine the specificity of the Fos-ir and ERα-ir labeling. The ventromedial hypothalamus (VMN; –2.45 mm; plate 28) contains neurons expressing ERα but is not facilitatory for maternal behavior [39] and, therefore, was not expected to contain many Fos-ir neurons in either group of dams [24]. The nucleus accumbens (NA; +0.95 mm; plate 14) does not contain ERα but is necessary for maternal retrieval and licking of pups [40] and was expected to contain many Fos-ir neurons only in stimulated dams [24]. The medial habenula (MHb; –3.90 mm; plate 32) does not contain ERα and is unnecessary for maternal behavior [11] and was not expected to show immunoreactivity for either the ERα or Fos proteins [24].

Fluorescent labeling was visualized with a Nikon Optiphot 2 microscope using filters for cyanine and fluorescein. Identical microscopic fields (370 × 500 μm) were captured two times at 10× with NIH Image, once with each filter. Single and dual-labeled Fos-ir and ERα-ir neurons were revealed by comparisons between photographs of the same microscopic field. Only neurons with brightly fluorescent labeling were counted. One section per site was chosen for each subject for analysis and one microscopic field from each section was analyzed within each hemisphere.

Data Analyses

One pup-stimulated dam was poorly perfused and removed from the study (resulting n = 9). The inability to find accurate histological matches resulted in the absence of immunocytochemical data from one nonstimulated dam within the LHb and LS_l (not the same subject; resulting n. for these sites = 8). In addition, within each BST site, we were unable to histologically match and collect data from 2 nonstimulated dams (resulting n. for this site = 7) and one pup-stimulated dam (resulting n. for this site = 8). Immunocytochemical data are expressed as the summed number of immunoreactive cells in both hemispheres. There was no effect of immunocytochemical run in the total number of ERα-ir neurons or Fos-ir neurons revealed across

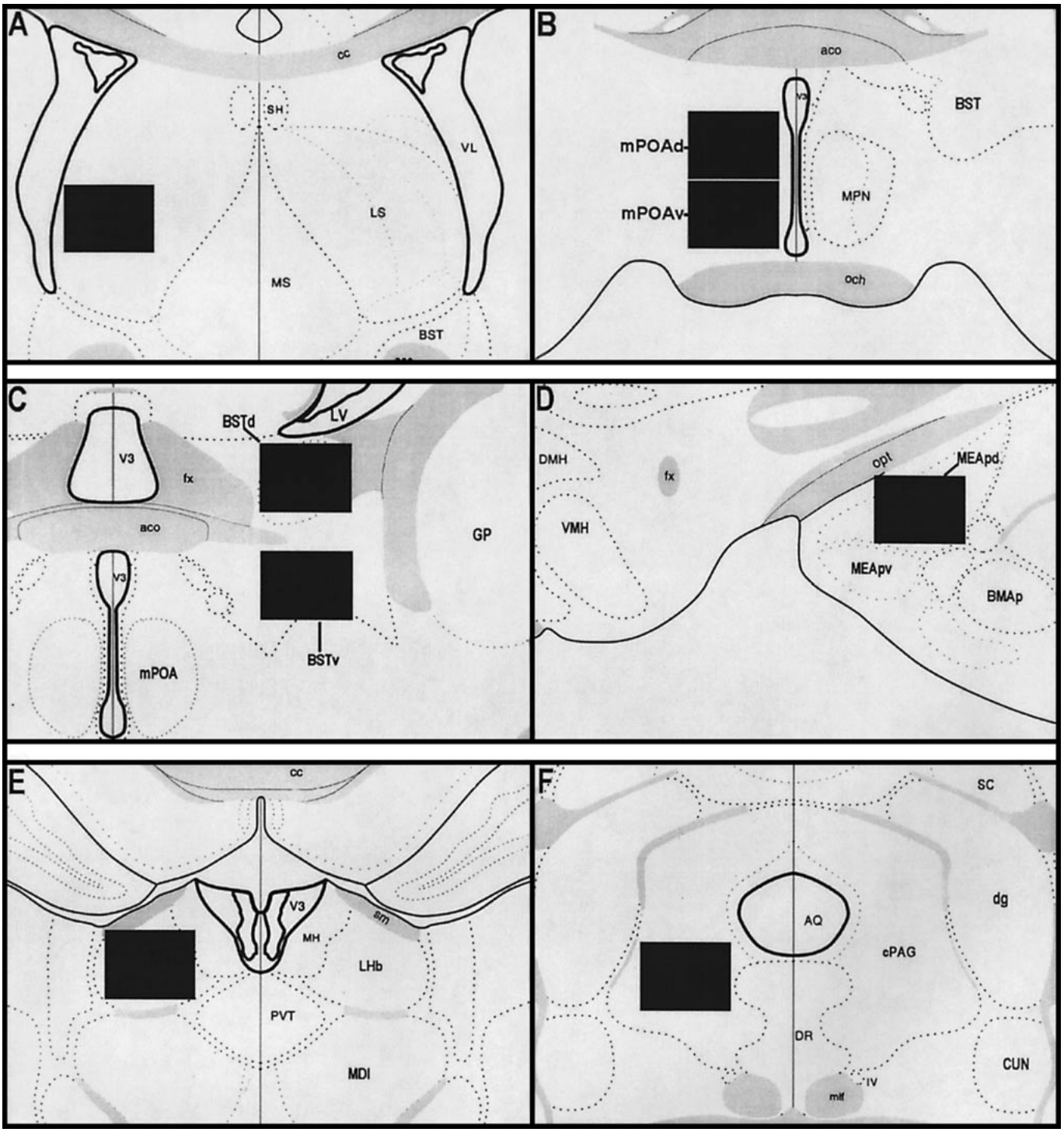


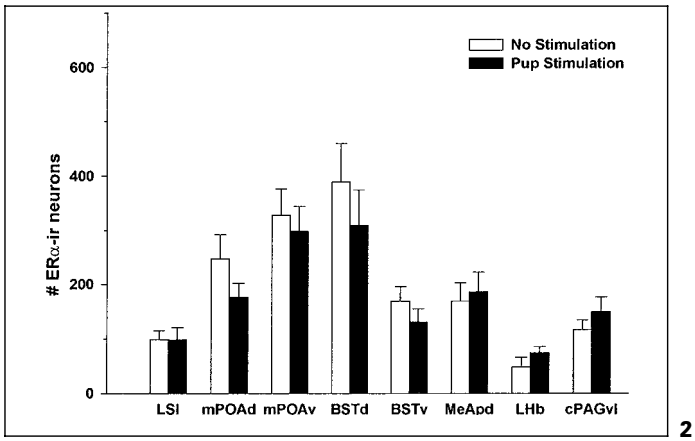
Fig. 1. Diagrammatic representation of neural sites (arranged rostrocaudally) that were analyzed microscopically for Fos-ir and ERα-ir in lactating dams. **A** Lateral septum. **B** Medial preoptic area. **C** Bed nucleus of the stria terminalis. **D** Posterodorsal medial amygdala. **E** Lateral habenula. **F** Caudal periaqueductal gray. Field of analysis is indicated by black rectangle. aco = Anterior commissure; AQ = cerebral aqueduct; BMAp = posterior basolateral amygdala; BST = bed nucleus of the stria terminalis; cc = corpus callosum; CUN = cuneate nucleus; dg = deep gray layer of the superior colliculus; DMH = dorsomedial hypothalamus; DR = dorsal raphe nucleus; fx = fornix; GP = globus pallidus; LHb = lateral habenula; LS = lateral septum; MEApd = posterodorsal medial amygdala; MEApv = posteroventral medial amygdala; MDI = mediodorsal thalamus; MH = medial habenula; mlf = medial longitudinal fasiculus; mPOAd = dorsal medial

groups and neural sites (all $p \geq 0.1$), and this factor was therefore not included in data analyses. All immunocytochemical data were first tested for the assumptions of normality and homogeneity of variance. If the data passed both tests, then the data from each neural site were analyzed with independent t tests. If the data failed either test, they were analyzed nonparametrically with Mann-Whitney U tests.

Results

The nine pup-stimulated dams included in the study were highly parental upon reunion with pups and the details of their behavior can be found elsewhere [29]. Dams quickly made physical contact with pups, soon thereafter retrieving most or all of their litter to the nest site, hovered over them while licking and self-grooming, and displayed prolonged periods of quiescent nursing behavior most often in the kyphotic posture. All litters interacting with dams showed at least one stretch response to milk letdown and all but one litter gained weight during the observation period. Although non-stimulated dams were not continuously observed, periodic spot checks indicated that they were generally inactive within a few minutes after removal and replacement of their cage top, as reported previously [24].

The total number of ERα-ir neurons in each of the eight experimental brain sites analyzed was similar between pup-stimulated and nonstimulated dams (all $p \geq 0.2$; fig. 2). Dams interacting with pups had significantly more Fos-ir neurons in all eight sites compared with nonstimulated controls (fig. 3). More than twice as many Fos-ir neurons were found for maternally behaving dams in the LS_l ($t = 4.12$, d.f. = 15, $p \leq 0.001$) and LHb ($t = 3.48$, d.f. = 15, $p \leq 0.004$), an approximately 4- to 6-fold increase was found in the BST_d ($t = 6.73$, d.f. = 13, $p \leq 0.0001$), MeA_{pd} ($U = 45.0$, d.f. = 16, $p \leq 0.0001$), $cPAG_{vl}$ ($t = 5.73$, d.f. = 16, $p \leq 0.0001$), $mPOA_d$ ($t = 4.17$, d.f. = 16, $p \leq 0.0007$) and $mPOA_v$ ($U = 45.0$, d.f. = 16, $p \leq 0.0001$). An almost 7-fold increase was found in the BST_v ($U = 28.5$, d.f. = 13, $p \leq 0.0003$).



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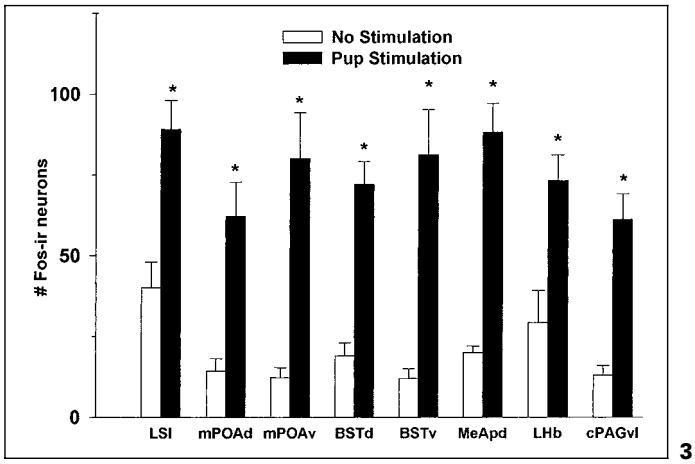

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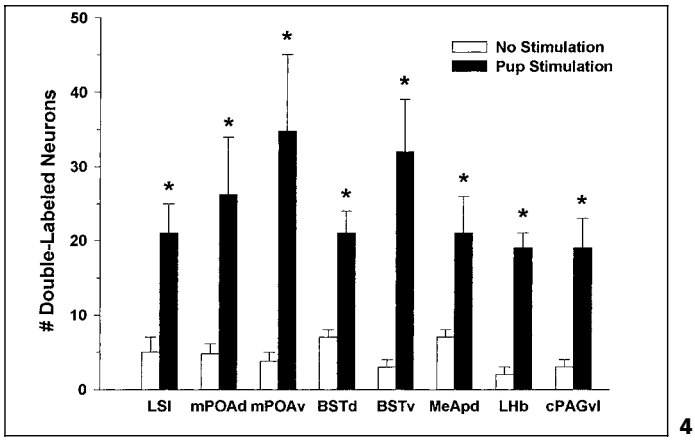

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Fig. 2. Number (mean ± SEM) of ERα-ir neurons in neural sites analyzed (arranged rostrocaudally) in lactating dams receiving no stimulation or pup stimulation for 60 min on day 7 postpartum.

Fig. 3. Number (mean ± SEM) of Fos-ir neurons in neural sites analyzed in lactating dams receiving no stimulation or pup stimulation for 60 min on day 7 postpartum. * $p \leq 0.05$.

Fig. 4. Number (mean ± SEM) of double-labeled neurons (Fos-ir and ERα-ir) in neural sites analyzed in lactating dams receiving no stimulation or pup stimulation for 60 min on day 7 postpartum. * $p \leq 0.05$.

preoptic area; mPOAv = ventral preoptic area; MPN = medial preoptic nucleus; MS = medial septum; och = optic chiasm; opt = optic tract; PVT = paraventricular thalamic nucleus; SC = superior colliculus; SH = septohippocampal area; sm = stria medullaris; V3 = third ventricle; VMH = ventromedial hypothalamus; VL = lateral ventricle; IV = fourth cranial nerve. Modified from Swanson [37].

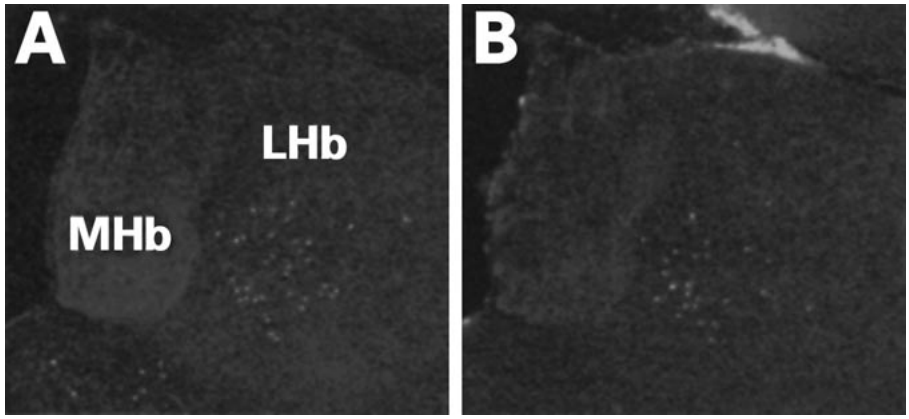

Fig. 5. Low magnification (4×) photomicrographs of **A** ERα-ir and **B** Fos-ir cells in the LHb of a representative dam that received pup stimulation. MHb = Medial habenula.

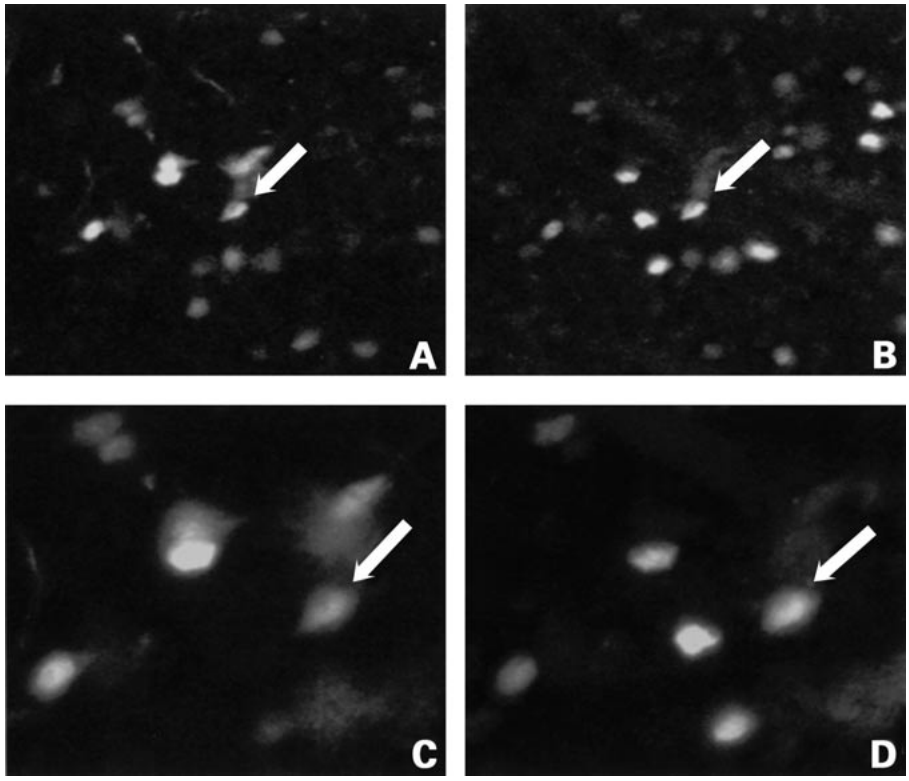

Fig. 6. 10× (**A**, **B**) and 20× (**C**, **D**) magnification photomicrographs of ERα-ir neurons (**A**, **C**) and Fos-ir neurons (**B**, **D**) in the LHb of a representative dam that received pup stimulation. The white arrow points to a neuron that contains both Fos-ir and ERα-ir.

The number of neurons containing both Fos immunoreactivity and ERα immunoreactivity was significantly greater for maternally behaving dams than nonstimulated dams in all eight sites (fig. 4). Indeed, dams interacting with pups had approximately 3–4 times more neurons containing both proteins compared with controls within the BST_d ($t = 4.76$, d.f. = 13, $p \leq 0.004$), MeA_{pd} ($U = 54.5$, d.f. = 16, $p \leq 0.007$), and LS_l ($t = 3.64$, d.f. = 15, $p \leq 0.003$), and 5- to 8-fold more Fos-ir plus ERα-ir neurons within the $cPAG_{vl}$ ($U = 51.0$, d.f. = 16, $p \leq 0.003$), $mPOA_d$ ($U = 55.0$, d.f. = 16, $p \leq 0.009$), and LHb ($U = 37.0$, d.f. = 15, $p \leq 0.0001$; fig. 5, 6). Maternally behaving dams had more than 9 times more neurons containing both proteins compared with nonstimulated control dams in the $mPOA_v$ ($U = 50.0$, d.f. = 16, $p \leq 0.002$; fig. 7, 8) and 11 times more in the BST_v ($U = 28.0$, d.f. = 13, $p \leq 0.003$).

In nonstimulated dams, between 10 and 40% of Fos-ir neurons also contained ERα-ir; between 25 and 45% of Fos-ir neurons also contained ERα-ir in dams interacting

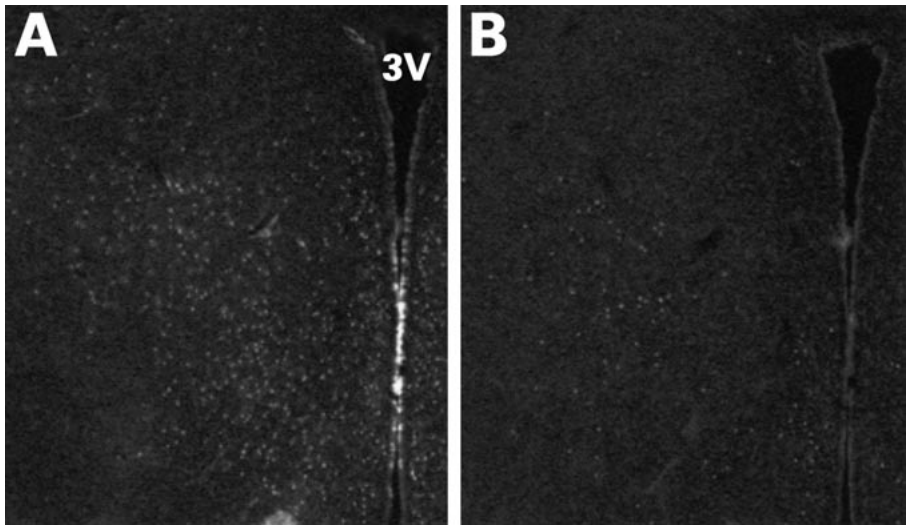

Fig. 7. Low magnification (4×) photomicrograph of **A** ERα-ir and **B** Fos-ir cells in the mPOA of a representative dam that received pup stimulation. 3V = Third ventricle.

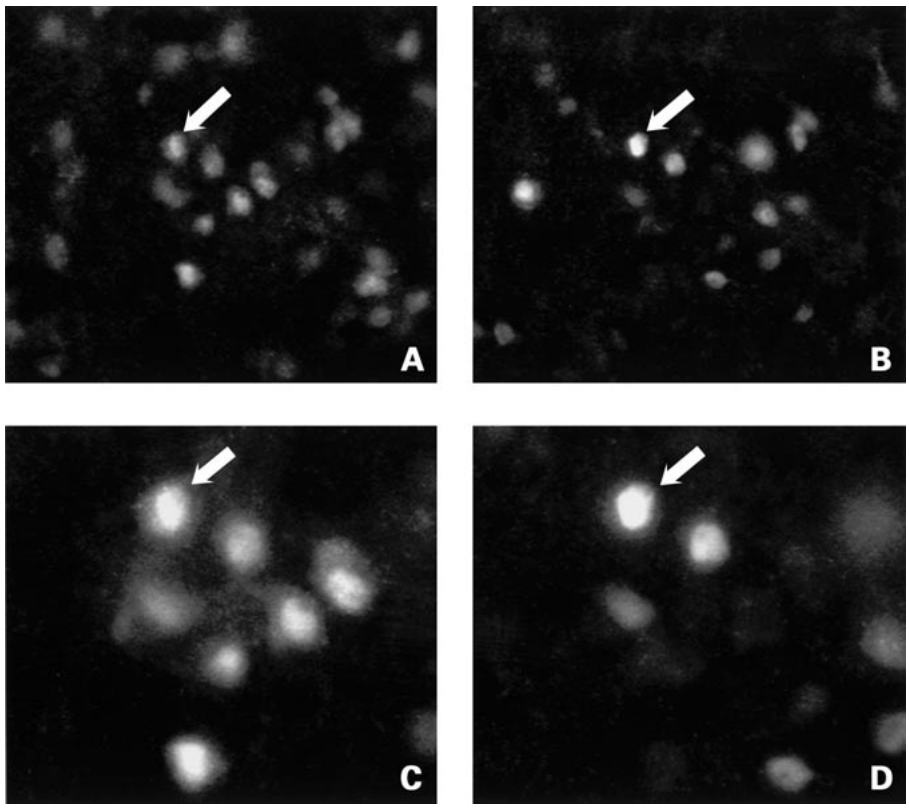

Fig. 8. 10× (**A**, **B**) and 20× (**C**, **D**) magnification photomicrographs of ERα-ir neurons (**A**, **C**) and Fos-ir neurons (**B**, **D**) in the $mPOA_v$ of a representative dam that received pup stimulation. The white arrow points to a neuron that contains both Fos-ir and ERα-ir.

with pups (fig. 9). Only within the LHb ($U = 47.5$, d.f. = 15, $p \leq 0.03$) and BST_v ($t = 2.71$, d.f. = 13, $p \leq 0.03$) was the percentage of double-labeled neurons greater in maternally-behaving than nonstimulated dams. In all other sites, the percentage of Fos-ir neurons that were also ERα-ir was similar between the two groups (all $p \geq 0.05$).

The three control sites displayed the expected presence or absence of immunoreactive labeling. The ventromedial hypothalamus (VMN) contained many ERα-ir neurons but very few Fos-ir neurons (≤5/hemisphere) for either group of dams. The nucleus accumbens contained many Fos-ir neurons for stimulated dams (≥30/hemisphere),

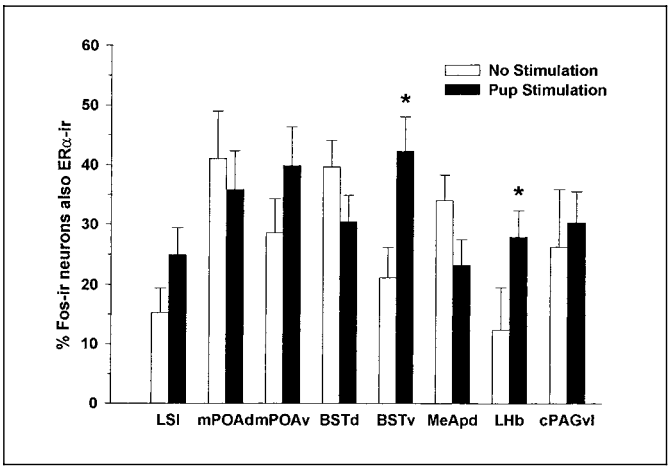

Fig. 9. Percentage (mean ± SEM) of Fos-ir neurons that were also ERα-ir in lactating dams receiving no stimulation or pup stimulation for 60 min on day 7 postpartum. * $p \leq 0.05$.

few for nonstimulated dams (≤ 5/hemisphere), and no ERα-ir neurons. Lastly, the medial habenula (MHb) contained neither Fos-ir nor ERα-ir neurons.

Discussion

In this experiment, we quantified the number of cells that co-expressed Fos and ERα after the display of maternal behavior in lactating rats one week after parturition. We found that within many sites that mediate maternal behavior, a substantial number (~25–45%) of cells that show increased Fos expression after interactions with pups also expresses ERα. The levels of dual-labeled neurons detected after maternal behavior in female rats are comparable to those found in some sites of the female rats brain after sexual stimulation (e.g. VMH and PAG) where many, but not the majority, of the Fos-ir cells are also ER-ir [31]. Although the functional significance of our findings awaits further research, they raise the possibility that ERα activity influences these cells even after the time when estradiol plays its role in the onset of maternal behavior.

Methodological Considerations

Immunofluorescent visualization of neural proteins may in some cases be less sensitive than some other visualization techniques and may result in an underestimation of the number of neurons expressing the protein under investigation [41]. Therefore, as in all experiments of this type, our results may underestimate the number of Fos-ir, ERα-ir, and double-labeled neurons in the brains of lactating rats after our experimental manipulations rather than provide an absolute number of neurons that contain these proteins. However, the relative increase in Fos-ir in the brains of maternally-acting dams compared with nonstimulated dams was very similar to that found in our previous studies using nonfluorescent visualization of Fos protein after maternal behavior [13, 22, 24]. Furthermore, regional differences in the relative density of ERα were also consistent with previous reports [15].

We believe that the co-expression of Fos-ir and ERα-ir is closely related to the performance of maternal behavior, and not primarily due to the chance that many Fos-ir neurons will be double-labeled if the neural site analyzed also contains a large number of cells expressing ERα. This is supported by our finding that the two sites with the greatest relative increase in dual-labeled cells – the vBST and LHb – did not have the largest number of either Fos-ir or ERα-ir cells. In fact, the LHb had the smallest number of ERα-ir cells of all the sites analyzed and one of the smallest increases in the number of Fos-ir cells in maternal dams compared with nonstimulated controls.

Neural c-fos Activity and Maternal Behavior

Consistent with previous reports, there was a significant increase in the number of Fos-ir nuclei in all eight experimental neural sites investigated after maternal behavior in lactating rats. Moreover, the approximately 4- to 5-fold increase in Fos-ir in the mPOA of maternal dams is comparable to the increases found in dams interacting with the pups either during parturition [42–44], one day later [20, 21], after 4–5 days of maternal experience [24, 26, 27], or after 8–11 days of experience with pups [25]. This may indicate that many of the neural populations that are genomically activated during the periparturitional onset of maternal behavior are similarly active during the display of maternal behavior throughout lactation. The absence of an increase in the number of Fos-ir nuclei in the MHb and VMH, areas where neural activity is not necessary for the facilitation of this behavior [11, 39, 45], has also been previously demonstrated [21, 24, 25, 46].

Lactating rats require the appropriate sensory stimulation from pups in order to display maternal behavior [3]. Although pups offer a variety of distal cues (olfactory, auditory, visual) that may have a small influence on the dam's parental responsiveness, the somatosensory cues that they provide are critical for the dam's maternal behavior [3], as well as for increased neural Fos expression. Dams that receive only distal stimulation from pups

do not show greater numbers of Fos-ir cells in any of the sites that we investigated compared with dams physically interacting with a non-pup stimulus [13, 24, 26]. This suggests that direct contact with pups and the performance of maternal behavior, but not distal stimulation from pups or general physical activity, stimulate high levels of Fos expression in these areas. In support of this interpretation, most of the neural sites we investigated that showed increased Fos-ir after mother-litter interactions are electrophysiologically responsive to peripheral tactile input [mPOA: 47; BST: 47, 48; cPAG: 49, 50; LHb: 51; LS: 52]. Furthermore, there is differential somatosensory regulation of *c-fos* activity in some sites of the maternal rat brain. Whereas suckling induces high levels of Fos expression in the $cPAG_{vl}$, a region necessary for the sensorimotor integration of suckling-induced kyphosis but not other maternal behaviors [13, 14, 22], it has no effect on the number of Fos-ir cells in the mPOA and BST_v [24, 26, 46], sites critical for some oral maternal activities such as retrieval [2].

Neural ER and Maternal Behavior

Of the two identified estrogen receptor subtypes (α and β), we chose to investigate the distribution of neurons that contain Fos-ir and ERα-ir because ERα is found in high abundance within sites necessary for maternal behavior [15]. Furthermore, studies of mice without functional ERα (ERα knockouts) suggest the necessity of ERα for many of estradiol's effects on reproductive physiology and behavior [53–56]. In contrast, the β form of the ER is not essential for reproductive behaviors, at least in mice [57]. Although the influence of the high levels of gestational estrogens on maternal behavior in Sprague-Dawley rats has likely waned by the end of the first week postpartum [58–60], we found that all eight neural sites analyzed in the brains of lactating rats on day 7 postpartum had a large number of cells that expressed ERα-ir. This is not surprising, considering that the primary antibody that we used recognizes both occupied and unoccupied ERα and that in numerous areas of the brain the total amount of estrogen receptors (occupied and unoccupied) changes only minimally in response to natural or experimental changes in circulating gonadal hormones [61–64]. In contrast, the number of cells in many neural sites that express progestin receptors changes dramatically through gestation and early lactation [65]. Distinct types of steroid hormone receptor regulation during pregnancy and postpartum could reflect the different ways that activation of these receptors influence maternal behavior.

Significance of Co-Expression of c-fos *and ERα for Maternal Behavior*

The mechanisms by which the display of maternal behavior is maintained postpartum by tactile stimulation from pups is not known. One possibility is that while estradiol is not necessary for postpartum maternal responsiveness, ERα may be. This is supported by the presence of ERα in many Fos-ir cells during mother-litter interactions, coupled with recent findings in other systems that steroid hormone receptors can be activated in the absence of ligand. For example, stimulation of the progestin receptor by mating even in the absence of endogenous progesterone may influence sexual behavior in female rats [66]. Dopamine-stimulated activity within progestin receptor-containing neurons may mediate these ligand-independent neural and behavioral effects [67, 68]. Similarly, the estrogen receptor can also be activated in a ligand-independent manner [69–72] by dopamine [73]. Because mesolimbic dopamine release is important for the display of active maternal behaviors, including retrieval and licking of pups [74–80], perhaps dopamine release in response to tactile stimulation from pups activates ERα in a subpopulation of neurons to influence maternal responsiveness during the postpartum period. This may be particularly true in sites such as the LHb and $mPOA_v$ that had a relatively high percentage of Fos-ir cells that were also ERα-ir. Direct interactions between the *c-fos* and ERα genes may contribute to this process, since an estrogen receptor response element is found on the *c-fos* gene [81–83]. This is supported by the finding that estradiol increases *c-fos* activity in brain sites such as the mPOA that are involved in the onset of maternal behavior [84, 85]. Our results may also be relevant for the periparturitional onset of maternal behavior, revealing a network of neurons that receive both hormonal and sensory information. Indeed, the behaviors expressed at the onset of maternal responding and the sensory stimuli that elicit them are the same throughout lactation, and estradiol and other hormones presumably facilitate the onset of maternal behavior by enhancing sensitivity to stimuli emanating from pups [3, 18].

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