

**From page 9: ...and because the major adrenomedullary hormone, epinephrine, does not cross the blood-brain barrier...**

## **Regulation of Reproductive Hormones**

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# **c-fos Expression in the Forebrain after Mating in the Female Rat Is Altered by Adrenalectomy**

Nicole Cameron Mary S. Erskine

Department of Biology, Boston University, Boston, Mass., USA

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Mary Erskine

Department of Biology, Boston University

5 Cummington St.

Boston, MA 02215 (USA)

Tel. +1 617 353 2093, Fax +1 617 353 6340, E-Mail [erskine@bu.edu](mailto:erskine@bu.edu)

## **ABC**

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## **Key Words**

*fos* w Adrenalectomy w Sexual behavior w Amygdala w Adrenal steroids w Arcuate nucleus w Paraventricular nucleus w Preoptic area

## **Abstract**

In rats of both sexes, mating stimulates neuronal activity in forebrain areas that are also activated by stress. Hypothalamic cells in the arcuate (ARC) and paraventricular (PVN) nuclei synthesize hormones or peptides whose levels are altered by adrenalectomy. In this experiment, we examined whether the mating-induced expression of *c-fos* in the forebrain is altered by adrenalectomy (Adx) in female rats. Ovariectomized females were adrenalectomized (Adx) or sham-operated (Sham), hormoneprimed and mated 2 weeks after surgery. They received 15 intromissions (15I), 5 intromissions (5I) or 15 mounts without intromission (MO) from a male or were taken directly from their home cage (HC). Two hours after mating, rats were perfused with paraformaldehyde and their brains were collected and stained immunocytochemically for FOS protein. FOS-immunoreactive (FOS-IR) cells in the posterodorsal medial amygdala (MePD), bed nucleus of stria terminalis (BNST), ventromedial hypothalamus (VMH), medial preoptic area (mPOA), ARC and PVN were counted bilaterally. In Sham animals, intromissions produced significant increases in FOS above HC levels. In Adx animals, mating increased FOS activity in all areas. However, responses to 5I and 15I differed between Sham and Adx groups. In all areas, Shams showed either the highest FOS response following 15I or levels which were

equivalent after 5I and 15I. In Adx animals, the greatest number of FOS-positive cells occurred after 5I, with the 15I group showing significant suppression of FOS below 5I levels in the VMH, mPOA, ARC and PVN. These results demonstrate that the adrenal modulates FOS responses to mating in the female rat and suggest that adrenal secretory products normally may decrease sensitivity to low levels of mating stimulation. These effects may be due to increased corticotropin-releasing hormone (CRH) or  $\beta$ -endorphin in the hypothalamus after adrenalectomy.

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### **Introduction**

In the female rat, stimuli received during mating initiate neuroendocrine and behavioral changes important for reproductive success. The fact that both the frequency and the temporal characteristics of intromission are important for the establishment of pregnancy has been well documented [1–4]. These two factors have been useful in identifying particular brain areas which may be involved in pregnancy initiation, because they influence the induction of immediate-early gene expression, an indicator of neuronal activation in some brain areas. Areas including the posterodorsal division of the medial amygdala (MePD), the posteromedial bed nucleus of stria terminalis (BNST), the medial preoptic area (mPOA) and the ventrolateral portion of the ventromedial nucleus of the hypothalamus (VMH) showed increases in the number of FOS-IR cells after mating [5–9] or artificially produced mechanical vaginocervical stimulation (VCS) [8–10]. Of these areas, the MePD appears particularly responsive to mating stimulation, showing increases with the number [6] and timing [11] of intromissions from males. Brain areas activated by mating have been shown to be important in the regulation of mating-induced, as well as stress-induced, prolactin (PRL) secretion [12–17]. The BNST, MePD, PVN, ARC and mPOA are known for their involvement in hypothalamic-pituitary-adrenal axis (HPA) responses to stress [18–20] and have been shown to be activated by mating [6, 21, 22]. In addition, stress has been shown to alter PRL release in ovariectomized estrogen-treated rats by increasing its plasma level when applied in the morning, when PRL levels are low, and by suppressing it when applied in the afternoon, when the plasma PRL levels are high [23, 24]. Furthermore, exposure to stress may be accompanied by disruption of reproductive function [25–27] including decreases in plasma PRL levels when applied during one of the two daily PRL surges expressed during early pregnancy [24, 28]. Although a relationship between the adrenal gland and reproductive function has been established [16, 29–31], there is no consensus about the role played by the adrenal gland or a clear understanding of the mechanisms by which adrenal hormones act on the reproductive system. Adrenal steroids may act directly in brain and/or pituitary to interfere with secretion of gonadotropins and PRL, or may act indirectly through neurochemical changes which

modulate pituitary hormone synthesis or release. Neurotransmitters involved in this way may include corticotropin-releasing hormone (CRH),  $\beta$ -endorphin and norepinephrine, all of which are known to be increased by stress [18, 32, 33]. CRH mRNA increases in the hypothalamus after adrenalectomy (Adx) [34], and CRH stimulates the pituitary release of  $\beta$ -endorphin [33] and PRL [32]. In turn, opioid peptides are known to be potent stimulators of PRL release [35–37]. Following Adx, elevations in opioid peptide ( $\beta$ -endorphin,  $\beta$ -endorphin-like and  $\Delta^3$ -MSH) concentration in the plasma and in anterior pituitary were shown to increase over time, reaching maximum levels 7–14 days after surgery [38]. In the present experiment, we have examined whether the induction of *c-fos* observed in the forebrain of female rats after mating is modulated by the adrenal gland.

### **Materials and Methods**

#### *Animals*

Experimental animals were female Long-Evans rats (Charles River Laboratories, Wilmington, Mass., USA) weighing 220–280 g. Experienced breeding males of the same strain (275–350 g) were used for all mating tests. Rats were housed singly in wire mesh cages under a reversed light cycle (lights on from 20.30 to 08.30) with food and water available ad libitum. Males and females were housed in the same animal room. All experimental procedures were approved by the Laboratory Animal Use and Care Committee at Boston University in accordance with NIH Guidelines.

#### *Surgery*

All females were ovariectomized via bilateral dorsal incisions under ketamine HCl (Ketaset, 95 mg/kg i.p.; Fort Dodge Labs, Fort Dodge, Iowa, USA) and xylazine (Rompun, 10 mg/kg, Bayer Co., Shawnee Mission, Kans., USA) anesthesia, and adrenal glands were removed from one half of the females (Adx,  $n = 20$ ) at the same time. In the other group, the adrenal glands were visualized but not removed (Sham,  $n = 21$ ). Adx animals were given a salt block in their cages for the remainder of the experiment to compensate for salt loss resulting from Adx.

#### *Procedures*

Twelve days after surgery, all experimental females received subcutaneous injections of estradiol benzoate (10  $\mu$ g/0.1 ml sesame oil s.c.) followed 48 h later by progesterone (500  $\mu$ g/0.1 ml sesame oil s.c.). Mating tests were carried out 4 h after progesterone treatment. Behavioral treatments were administered in a dimly illuminated room between 11.30 h and 14.30 h. The sexually naive females were placed individually into glass testing chambers (30 ! 26 ! 50 cm) with a sexually experienced male. Groups of females received 5 intromissions (5I), 15 intromissions (15I), 15 mounts without intromission (mounts only, MO), or were not exposed to males (home cage, HC). Females receiving MO treatment were fitted with vaginal masks made of cloth tape before mating as previously described [6]. Animals in the two HC groups (Sham and Adx) were not exposed to males or the testing room. During each test, the occurrence and intensity (lordosis rating; LR) [39] of lordosis, and the timing of each mount without intromission, mount with intromission, and ejaculation received by the female was recorded. The percentage of times that a female showed lordosis in response to a mount (lordosis quotient, LQ) and the mean LR were calculated. The mean intromission interval (II; seconds between intromissions) was derived from the timing data [5].

#### *Perfusions and Immunocytochemistry*

Two hours after receiving the first mount or intromission, females were deeply anesthetized with sodium pentobarbital (Somlethal, 120 mg/kg). HC animals were anesthetized immediately after

removal from their home cage at this time. Before perfusion a cardiac blood sample was taken for later determination of serum corticosterone levels. Blood samples (0.3 ml) were centrifuged at 4°C at 2,000 rpm for 20 min and stored at -20° C until assay. Perfusion was carried out intracardially as previously described [7]. Sodium heparin (100 U/0.1 ml) was injected into the left ventricle before perfusion with 250 ml of phosphate-buffered saline (0.1M PBS, pH = 7.2) followed by 250 ml of 4% paraformaldehyde. Brains were then removed and postfixed in 4% paraformaldehyde for 1h. Adrenalectomy and Mating-Induced FOS Neuroendocrinology 2003;77:305-313 307 followed by cryoprotection in a 25% sucrose-PBS solution. Thirty-micrometer sections were cut through the hypothalamus and preoptic area using a freezing microtome. They were subsequently stored at -20°C in anti-freeze solution made of 0.05 M Tris-buffered saline (pH 7.6), 1% polyvinyl-pyrrolidone and 30% ethylene glycol. Every third section was used for FOS immunocytochemistry. For immunocytochemistry, free-floating sections were washed with PBS and then incubated for 24 h at room temperature on a shaker with the primary anti-FOS antiserum (sc-52, Santa Cruz Biotechnology, Inc.) diluted at 1:1,000 in a 0.1 M PBS solution containing 0.1% sodium azide and 0.4% Triton X-100. Sections were then incubated in 1% hydrogen peroxide and 3% normal goat serum in PBS. Visualization of FOS-IR was carried out using biotinylated anti-rabbit IgG (Vector Labs, Burlingame, Calif., USA) diluted at 1:200 in 0.4% Triton X-100 PBS, the Elite Vectastain avidin-biotin horseradish peroxidase kit, and 3,3'-diaminobenzidine (Vector Labs) as the chromogen. Sections were mounted onto gelatin-coated slides, dehydrated in ethanol, cleared in xylenes and coverslipped with Permount. The numbers of FOS-IR cells were counted bilaterally in one section from the MePD, BNST, PVN, ARC, VMH and mPOA. Slides were coded by an independent observer so that sections were counted without knowledge of group assignment. A quadrilateral template (mPOA, PVN, ARC and BNST: 460 ! 470 !m; MePD: 510 ! 230 !m; VMH: 350 ! 350 !m) was superimposed over each area at 200! magnification using a camera lucida. These templates were designed to fully encompass the areas of interest. FOS-IR cells within that template were transcribed onto data sheets and counted. The number of FOS-IR cells counted on both sides was taken for each animal. In some cases, the numbers of animals were different from the total number of animals in the experiment because staining of some brain areas was unsuccessful.

#### *Verification of Adx*

The level of serum corticosterone was used to attest to the success of the Adx. Serum was assayed for corticosterone using a RIA kit (ImmuChem Double Antibody, ICN Biomedica, Inc. Costa Mesa, Calif., USA) and Adx animals showed a mean serum corticosterone concentration of 21.7 B 3.6 ng/ml and had a mean body weight loss of 24.0 B 4.5 g between the time of surgery and the day of testing (n = 12). In contrast, the mean serum corticosterone concentration of the Sham animals was 563.9 B 20.9 ng/ml and for the same period, they did not change their body weight (0.0 B 3.4 g; data available only for 4 animals).

#### **Results**

The number of FOS-IR cells present in the MePD, BNST, VMH, mPOA, ARC and PVN were quantified within standard areas as indicated in figure 1. Two-way ANOVA (surgical treatment ! mating treatment) revealed a significant overall effect of mating treatment in every brain area investigated. Also in every brain area, levels of FOS-IR in the HC group were similar in the Sham and Adx treatment groups, suggesting that constitutive *c-fos* expression was not altered by Adx.

**Fig. 1.** Areas in which Fos-IR cells were quantified. Rectangles indicate position and size of templates. Cell counts were made bilaterally

in each section. Numbers indicate the level of section according to Paxinos and Watson [1].

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**Fig. 2.** Fos-IR cells (arrows) in Sham 5I and Adx 5I groups in the medial amygdala.

**Fig. 3.** Mean number of Fos-IR cells (BSEM) obtained in the 8 groups in the MePD (a) and BNST (b). Statistically significant differences were observed between bars labeled with different letters; bars labeled with the same letter were not significantly different from each other. Duncan's post hoc tests were significant at  $p < 0.05$ .

Photomicrographs of representative FOS-positive cells in the Sham 15I and Adx 15I in the MePD are shown in figure 2. Dark nuclear staining characteristic of FOS labeling was observed, and no staining was observed in the absence of the primary FOS antibody.

Mean numbers of FOS-IR cells present in the MePD and BNST are shown in figure 3. In the MePD (fig. 3a), a significant overall effect of mating treatment [ $F(3, 33) = 13.57, p < 0.001$ ] followed by post hoc analysis using Duncan's test revealed that Sham females showed significantly greater mean numbers of FOS-IR cells in the 15I and 5I groups than in the HC and MO groups ( $p < 0.05$ ). In contrast, Adx females receiving 5I but not 15I showed significantly greater numbers of FOS-IR cells in the MePD than both the HC and MO groups ( $p < 0.05$ ). The number of FOS-IR cells present in the MePD in the Adx 15I group did not differ from any other Adx group. Although Adx and Sham animals showed equivalent responses to 5I, the response to 15I differed between surgical treatments; mean numbers of FOS-positive cells after 15I were significantly greater in Sham animals than in Adx animals in this brain area ( $p < 0.05$ ).

A significant effect of mating treatment [ $F(3, 33) = 4.80, p < 0.007$ ] was seen in the BNST (fig. 3b). In this nucleus, the Sham 15I group but not the 5I and MO groups showed mean numbers of FOS-IR cells which were significantly elevated over Sham HC levels ( $p < 0.05$ ). In Adx animals, only the 5I group showed a significant increase above the HC group ( $p < 0.05$ ). There were no significant differences between the Sham and Adx 15I, MO and HC groups.

Mean numbers of FOS-IR cells present in the VMH and mPOA are shown in figure 4. Significant effects of mating treatment [ $F(3, 28) = 22.89, p < 0.001$ ] and an interaction between surgery and mating treatment [ $F(3, 28) = 10.41, p < 0.001$ ] were seen in the VMH (fig. 4a). Post hoc tests for Sham animals revealed that the 15I and MO groups had significantly greater numbers of Adrenalectomy and Mating-Induced FOS *Neuroendocrinology* 2003;77:305–313 309

**Fig. 4.** Mean number of Fos-IR cells (BSEM) obtained in the 8 groups in the VMH (a) and mPOA (b). Statistically significant differences were observed between bars labeled with different letters; bars labeled with the same letter were not significantly different from each other. Duncan's post hoc tests were significant at  $p < 0.05$ .

**Fig. 5.** Mean number of Fos-IR cells (BSEM) obtained in the 8 groups in the ARC (a) and PVN (b). Statistically significant differences were observed between bars labeled with different letters; bars labeled with the same letter were not significantly different from each other. Duncan's post hoc tests were significant at  $p < 0.05$ .

FOS-IR cells than the 5I and HC groups in this area ( $p < 0.05$ ). The Sham 5I group had significantly greater numbers of FOS-IR cells than the HC group. The response to mating seen in Adx animals in this nucleus was different from that in Sham animals. The Adx 15I group had significantly greater numbers of FOS-IR cells than the Adx MO and HC groups ( $p < 0.05$ ), but the 5I group had significantly greater numbers of FOS-IR cells than any other Adx group, including the 15I group ( $p < 0.05$ ). Activity increased with MO in both Sham and Adx animals, but the Sham 5I group showed a relatively modest increase compared to that in the Adx 5I group. The FOS response to mating in the 15I Adx group was significantly lower compared to the 5I Adx group ( $p < 0.05$ ).

A significant effect of mating treatment [ $F(3, 31) = 8.72, p < 0.001$ ] and an interaction between surgery and mating treatment [ $F(3, 31) = 6.11, p < 0.002$ ] also were seen in the mPOA (fig. 4b). For this nucleus, post hoc tests revealed that the Sham 15I group had significantly greater numbers of FOS-IR cells than did the Sham HC group ( $p < 0.05$ ). The Sham 5I and MO groups were intermediate and not significantly different from the other groups. In Adx animals, the 5I group again showed a number of FOS-IR cells greater than any other Adx group ( $p < 0.05$ ). As in the VMH, activity was lower in the Adx 15I group compared to the 5I group ( $p < 0.05$ ). The 15I group was not significantly greater than Adx MO and HC groups nor did it differ from any Sham group.

In the ARC, significant effects of mating treatment [ $F(3, 28) = 3.70, p < 0.023$ ] and an interaction between surgery and mating treatment [ $F(3, 28) = 3.61, p < 0.025$ ] were seen (fig. 5a). Post hoc tests revealed that the only

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**Table 1.** Number of mounts, lordosis quotient (LQ), lordosis rating (LR) and inter-intromission-interval (III) in Sham and Adx female rats during mating (mean  $\pm$  SEM)

Treatment	Mounts <sup>a</sup>	LQ	% LR	III, s
Sham MO	14.7	0.2	96.4	2.9
Adx MO	15.8	0.7	92.5	1.2
Sham 5I	5.1	1.2*	92.4	6.3
Adx 5I	14.0	3.3	97.8	2.2
Sham 15I	14.8	4.2	93.4	4.7
Adx 15I	14.8	3.2	94.0	1.7

\*  $p < 0.05$ , significantly lower than all other groups on this measure.

<sup>a</sup> Mean frequency of mounts without intromission.

group showing a statistically significant increase was the Adx 5I group which showed a mean number of FOS-IR cells greater than all the other Sham or Adx groups ( $p < 0.05$ ). The mean number of FOS-IR cells seen in the Adx 5I group was approximately three times greater than all the other groups.

Mean numbers of FOS-IR cells present in the PVN showed significant effects of mating treatment [ $F(3, 33) = 11.48, p < 0.001$ ] and an interaction between surgery and mating treatment [ $F(3, 33) = 3.11, p < 0.04$ ] (fig. 5b). In contrast to all other brain areas examined, the Sham MO group showed a greater number of FOS-IR cells than Sham 15I and HC groups ( $p < 0.05$ ). In the Sham MO and

5I groups in the PVN, the elevation of FOS-IR cells was significantly greater than in the Sham HC group ( $p < 0.05$ ). In Adx animals, the response was different. Adx MO and 15I groups were not significantly different from the Adx HC group. The group showing the greatest number of FOS-IR cells in this nucleus was the 5I group ( $p < 0.05$ ), which was significantly greater than all the other Adx groups.

Two-way ANOVA revealed no significant differences between groups in III, LR, and LQ (table 1). The overall mean LQ, LR and III measures were  $94.3 \pm 2.3$ ,  $1.6 \pm 0.6$  and  $63 \pm 6.7$  s, respectively. The number of mounts without intromission in the Sham 5I group was significantly lower than in any other group ( $p < 0.05$ ).

### **Discussion**

In this study, we investigated the modulation by the adrenal gland of the induction of *c-fos* expression within forebrain areas after mating. Previous studies had shown that mating stimulation increased the number of FOS-IR cells present in the MePD, BNST, mPOA, VMH, PVN and ARC [3, 6, 9, 21, 22]. The MePD, BNST, mPOA, PVN and ARC have also been shown to be activated by stress [18–20, 40–42]. The results of this study have demonstrated that adrenalectomy affects mating-induced activation of FOS-IR cells in all brain areas examined except the BNST, and suggest that adrenal hormones may play a direct or indirect role in the expression of *c-fos* in the brain after mating.

The present results confirm previous data showing that mating with VCS induces *c-fos* expression in the hypothalamus and other limbic structures [3, 6, 9, 21]. As we previously showed, VCS induced a significantly greater number of FOS-IR cells in adrenal-intact animals than both the HC and MO control groups in the MePD [3, 6]. Previously, we have demonstrated that the MePD is particularly sensitive to variations in the characteristics of the mating stimuli received by the female rat [3], and have suggested that the MePD is important for transduction of the mating stimulation into the behavioral and neuroendocrine responses known to occur after VCS [3]. In the BNST, mPOA and VMH, 15I were needed to see significant FOS increases above HC levels, but these increases were not statistically different from the MO group. The lack of a significant increase over MO control levels after 15I differs from several previous reports [3, 6, 7, 21]. Our present findings could be the result of increased variability among the 5I and 15I animals due to differences in the numbers of mounts-without-intromission received (table 1), or other factors, such as hormonal status, time of testing, or differential handling procedures between experimenters. These factors have been shown previously to affect FOS responses to mating in the PVN [6], VMH [21, 43] and mPOA [3, 6, 9]. Consistent with previous studies [7, 8], FOS expression in the PVN and ARC of Sham animals did not reflect specific responses to VCS when mating occurred around the midpoint of the dark phase of the

light-dark cycle. However, FOS expression within PVN cells occurs within several distinct phenotypic classes, and further characterization of the FOS-IR cells is required before the effects of mating in Adx animals can be associated with activity within a particular subpopulation of PVN cells. Lastly, the labeling which we observed throughout the ARC did not differ between mated and Adrenalectomy and Mating-Induced FOS Neuroendocrinology 2003;77:305–313 311 nonmated groups in the Sham animals. Dopaminergic cells expressing FOS or FOS-related antigen within the dorsomedial ARC show a semicircadian rhythm [45, 46], and our behavioral testing occurred at a time when dopamine activity is high, potentially masking an effect of VCS in this area.

The pattern of *c-fos* expression induced by mating in the Adx females was different from that observed in the Sham animals. Adx groups showed clear responses to VCS, with increased levels of FOS-IR in the 5I group above MO levels in all the brain areas counted. These increases reached statistical significance for the MePD, VMH, mPOA, and PVN. Furthermore, the 15I group showed significantly lower numbers of FOS-IR cells compared to the 5I group in the VMH, mPOA, ARC and PVN. The Adx 5I group showed the greatest level of FOS expression in all the hypothalamic areas counted in this experiment. Not only were the responses significantly greater than other Adx groups, but in the mPOA, VMH and ARC, the mean numbers of FOS-IR cells after 5I were significantly greater than those in the comparable Sham group. It could be argued that the differential response shown by the Sham and Adx 5I groups was related to the stress of the VCS or testing conditions as demonstrated by the elevated level of corticosteroids detected at the time of perfusion, however if this were true, similar responses might have been expected among 5I and 15I animals in both treatment groups. Stress increases *c-fos* expression in many brain areas including the MePD, ARC, PVN and mPOA, though not in the VMH [20, 47]. Adx per se does not affect baseline *c-fos* expression [present study, 48, 49] or the FOS mRNA response to stress [50].

The most striking difference between Sham and Adx responses to mating was that Adx animals showed dramatic increases in FOS-IR after only 5I, while the Sham animals showed increases only in the 15I group. The observed increases in activity seen in the Adx 5I forebrain may reflect an altered peripheral and/or central sensitivity to the mating stimulus caused by the Adx, since 5I induced greater numbers of FOS-IR cells in Adx than in Sham animals in the VMH, mPOA, ARC and PVN. Another possibility is that Adx may have activated a mechanism whereby greater numbers of intromissions inhibit *c-fos* expression in the hypothalamus and mPOA. Dorsal horn cells located at the L6/S1 level of the spinal cord have been shown to be inhibited by mating [51, 52] and may be involved in induction of mating-induced analgesia via spinal CRH or opiateergic neurons [53, 54]. A

suppression of activity at the lumbar level may decrease hypothalamic sensitivity either through VCS-induced analgesia or through some other mechanism to reduce FOS responses to 15I in the Adx animals. In adrenalectomized animals, this decreased sensitivity to 15I may be prevented by effects of endogenous adrenal corticosteroids at spinal and/or hypothalamic levels. The difference between Sham and Adx animals in responsiveness to intromissions could explain the conflicting data regarding the effect of adrenalectomy on pregnancy rate in rats given varying amounts of genitosensory stimulation [55, 56]. We have recently demonstrated that adrenalectomy increases the likelihood of pregnancy in rats when the number of intromissions is low [Cameron, Ha and Erskine, unpubl. data].

A particularly strong effect of adrenalectomy was observed in the ARC nucleus where the Adx 5I group showed a significantly greater increase in FOS-IR than seen in the 5I Sham group. The ARC plays an important role in regulation of the HPA axis as well as PRL secretion, in part via  $\beta$ -endorphin neurons with the ARC [57]. Studies using intact estrous females showed an increase in FOS activity in  $\beta$ -endorphin neurons in the ARC after mating [21, 57] and Adx has been demonstrated to increase hypothalamic  $\beta$ -endorphin in males [60].  $\beta$ -Endorphin is known to be a potent stimulator of PRL release [35, 37, 58], and endogenous opioid peptides have been shown to modulate both the proestrus and the mating-induced prolactin surges in female rats [59]. Because corticosterone replacement returns opioids [60, 61] and PRL [62] to pre-Adx levels and because the major adrenomedullary hormone, epinephrine, does not cross the blood-brain barrier [63], we think that adrenocortical but not adrenomedullary secretions are responsible for the changes in *c-fos* expression observed in Adx animals. Further experiments are needed to determine if the FOS-IR cells in the ARC of Adx animals were, in fact,  $\beta$ -endorphin neurons, and whether Adx alters mating-induced increases in activity of  $\beta$ -endorphin neurons and PRL secretion in these animals.

The present results do not allow us to identify which of the several areas examined are important for processing of the sensory information coming from VCS or for neuroendocrine changes generated downstream from the MePD, and our ideas about the circuit(s) involved in this process must remain speculative. Because the adrenal modulated mating-induced activity in all areas examined, it is not possible to identify specific areas involved separately in reproduction and/or stress without further characterization of the activated cells. For instance, the results obtained in the PVN of Sham animals showed that activation was as high in the MO group as in groups receiving 5I and 15I. This suggests that activity seen in the PVN was not caused by VCS alone, but that other, possibly stressful, stimuli contributed to the FOS response. The strong

effects of Adx observed on mating-induced FOS responses in the forebrain nuclei measured may result from interactions between the reproductive and HPA axes which influence the ability of VCS to activate cells within the hypothalamus and preoptic area.

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