

Reproductively-Relevant Stimuli Induce Fos-Immunoreactivity within Progesterin Receptor-Containing Neurons in Localized Regions of Female Rat Forebrain

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Abstract

An experiment was conducted to determine if neurons that respond to stimuli associated with mating in female brain also contain progesterin receptors. We found that a portion of the neurons that respond to stimuli associated with mating also contains progesterin receptors. While the appropriate hormonal conditions are important for sexual receptivity, somatosensory information provided by the male also influences sexual behavior. One important stimulus provided by the male during copulation is vaginal-cervical stimulation (VCS). VCS has been shown to elicit many different behavioral and endocrine changes in female rats, such as increases in lordosis, pseudopregnancy, and termination of sexual receptivity. VCS also increases the expression of the immediate early gene product, Fos, in areas associated with reproduction. A portion of the neurons responding to VCS with increased Fos-immunoreactivity (Fos-IR) in female rat forebrain also contains estrogen receptors, illustrating that hormonal and mating-stimuli converge in a population of cells. As progesterone also plays an important role in female sex behavior, it is important to determine if some of the neurons also integrate information concerning serum progesterone levels and social interactions. Thus, we used a dual immunofluorescent technique to label both Fos-IR and progesterin receptor-immunoreactivity (PR-IR) in the brains of estradiol-primed, ovariectomized female rats following VCS manually applied by the experimenter. Many of the neurons that respond to VCS with increased Fos-IR within the medial preoptic area, the arcuate nucleus, and the progesterin receptor-rich areas of the rostral and caudal ventromedial nucleus of the hypothalamus also contain PR-IR.

During the estrous cycle of female rats, sexual behavior is dependent upon an increase in estradiol levels followed by a surge of progesterone (1–3). Reproductive behavior is abolished by ovariectomy and reinstated by the sequential treatment of estradiol followed one or more days later by progesterone. Although estradiol treatment alone can induce sexual receptivity, sequential treatment with estradiol and progesterone allows lower doses to be used (4), the onset and termination of reproductive behavior to be more predictable, and increases solicitation behavior in rats (5).

It is believed that many of the neuronal actions of steroid hormones on sexual behavior are mediated through intracellular steroid receptors (6), although some of the effects may be mediated by membrane receptors (7, 8). One mechanism by which steroid hormone receptors are activated is by a cognate ligand binding to the receptor. Treatments with drugs that block the binding of estradiol or progesterone to steroid receptors block the actions of steroid hormones on sexual behavior (6). In addition, intracerebroventricular infusions of antisense oligonucleotides directed at progesterin receptor mRNA, which block the synthesis of progesterin receptors, inhibit progesterone facilitation

of sex behavior (9). Activation of the steroid receptor by a ligand results in a conformational change that allows the steroid-receptor complex to bind to a hormone response element located on DNA (10, 11). Once the steroid-receptor complex binds to DNA, it can regulate gene transcription, therefore protein synthesis, and ultimately neuronal function (11, 12). Some cellular outcomes of estradiol and/or progesterone treatments are changes in second messenger systems (13), neurotransmitter/peptide receptor levels (14–16), neurotransmitter release (17), and immediate early gene *c-Fos* expression (18). Another outcome of estradiol treatment is the induction of progesterin receptors (19) within estrogen receptor-containing cells (20). Although progesterin receptors are found throughout the guinea pig and rat brain (19, 21), the highest concentrations of estradiol-induced progesterin receptors are found within the preoptic area, arcuate nucleus, and ventromedial hypothalamus (22, 23).

While the appropriate underlying hormonal conditions are important for sexual receptivity, mechanostimulation provided by the male also influences sexual behavior (24). One important stimulus provided by the male during mating is vaginal-cervical stimulation (VCS). VCS elicits many different behavioral and

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To determine if VCS influences progesterin receptor-containing neurons, a dual immunofluorescent technique was used to label both Fos and progesterin receptors in female rat brain following VCS manually applied by the experimenter.

Results

Consistent with previous reports, VCS dramatically increased Fos-IR within the medial preoptic area (MPOA), arcuate nucleus (Arc), and two levels of the ventromedial hypothalamus (VMH; $P < 0.05$, Fig. 2A). With the immunofluorescent technique described, PR-IR was observed only within the MPOA, Arc, and the VMH of both VCS+ and VCS- animals. While a small number of PR-IR cells was also seen in the posterodorsal medial amygdala in adjacent sections immunostained using a diaminobenzidine technique, no immunostaining of PR-IR was observed using immunofluorescence. In addition, the number of Fos-IR neurons is lower using immunofluorescence than that observed using diaminobenzidine. Thus, only the MPOA, the Arc, and the VMH were examined for colocalization of Fos-IR and PR-IR (Fig. 1A-C).

Within the MPOA, VCS increased the number of Fos-IR cells containing PR-IR by 150% ($VCS- 28.6 \pm SEM 5.22$, $VCS+ 71.5 \pm 15.26$; $P < 0.02$, Fig. 2). In the rostral ventrolateral ventromedial nucleus of the hypothalamus (rVMHVL), VCS induced a 50-fold increase in the number of Fos-IR cells containing PR-IR ($VCS- 0.5 \pm 0.43$, $VCS+ 25.0 \pm 3.76$; $P < 0.001$, Fig. 2B). In the ovarian steroid hormone receptor (i.e., estrogen receptor and progesterin receptor)-containing area associated with the rostral ventrolateral ventromedial nucleus of the hypothalamus that extends dorsally towards the fornix (rVMHVL-ORA; Fig. 3A), VCS induced more than a 50-fold increase in the number of Fos-IR cells colocalized with PR-IR ($VCS- 0.4 \pm 0.20$, $VCS+ 27.8 \pm 3.00$; $P < 0.001$, Fig. 2B and Fig. 3). Within the caudal VMHVL, VCS also induced a dramatic increase in the number of Fos-IR cells containing PR-IR ($VCS- 1.0 \pm 0.53$, $VCS+ 37.3 \pm 2.32$; $P < 0.001$, Fig. 2B and Fig. 4). Finally, in the Arc,

VCS increased the number of Fos-IR cells containing PR-IR by 170% ($VCS- 5.8 \pm 1.62$; $VCS+ 15.7 \pm 2.87$; $P < 0.02$, Fig. 2B).

Discussion

In agreement with previous studies (31, 32, 37-41), VCS increased dramatically the number of Fos-IR neurons within the MPOA, Arc, and the VMH (Fig. 2A). Due to lower sensitivity of the fluorescent technique, PR-IR was observed only within the MPOA, Arc, and the VMH, while in adjacent tissue sections that were reacted with diaminobenzidine some PR-IR cells were observed in the posterodorsal medial amygdala. Thus, only the MPOA, Arc, and two levels of the VMH were examined. It should be noted that, as the technique for identifying Fos-IR and PR-IR neurons was not optimal, the percentages of Fos-IR neurons containing PR-IR are likely to be underestimates. In addition, the concentration of Fos antibody used in the immunocytochemical procedure was titrated to observe relatively few Fos-IR neurons in the absence of VCS. However, the results demonstrate that some of the neurons that respond to VCS also contain progesterin receptors. In the MPOA, 31% of the cells that expressed Fos-IR after VCS also contained PR-IR, suggesting that there is a population of PR-IR neurons located in this area that is capable of responding to both progesterone and mechano-stimulation associated with mating.

VCS also induces Fos expression within PR-IR neurons in two levels of the VMH. We analyzed two rostral-caudal levels of the VMH, as the distribution of PR-IR neurons differs between these two levels. In the rVMHVL, the mean number of Fos-IR neurons containing PR-IR increased from 2% in VCS- controls to 31% in VCS+ animals. However, the PR-IR neurons within the rVMHVL are not confined to the nissl defined nucleus; rather, they extend dorsally towards the fornix. In this paper, we have defined the more inclusive ovarian steroid hormone receptor (i.e., estrogen receptor and progesterin receptor)-containing area associated with the rVMHVL as the rVMHVL-ORA (Fig. 1B). Within this neuroanatomical area defined on the basis of progesterin recep-

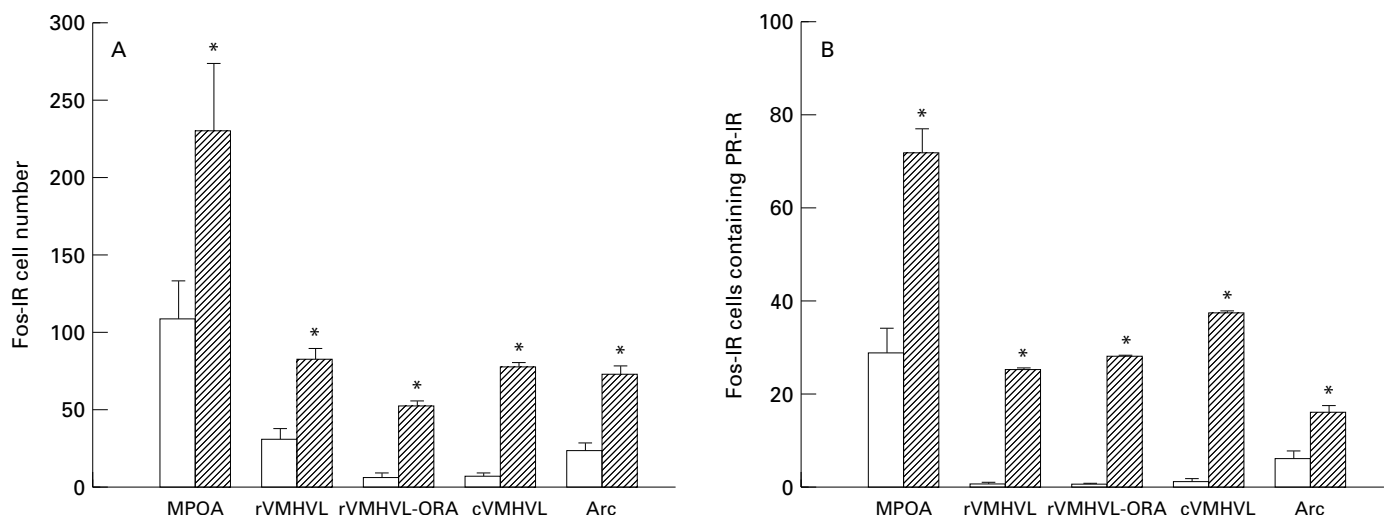


FIG. 2. A. Mean number (\pm SEM) of Fos-IR cells following vaginal-cervical stimulation (VCS+) or control perineum stimulation (VCS-). B. Mean number (\pm SEM) of Fos-IR cells containing PR-IR following \square VCS-; or ▨ VCS+.

to cytoarchitecturally defined regions. The effects of VCS on PR-IR neurons in these areas may increase lordosis (25, 26), induce pseudopregnancy (27), or terminate sexual receptivity (28).

In addition to the VMH, VCS increased the number of Fos-IR neurons in the Arc and to a small extent the number of Fos-IR neurons containing PR-IR (VCS- 5.8; VCS+ 15.7; Fig. 2B). Thus, mechanostimulation influences neurons containing progesterin receptors located within the MPOA, the VMH, and the Arc. Therefore, the data suggest that progesterin receptor-containing neurons located within these three areas are capable of integrating mechanostimulation provided by the male and information relating to serum progesterone levels. That is to say, the neurons may be influenced by both mating stimuli and progesterone. It should be restated that the percentages of colocalization are not absolute, especially since the fluorescent technique for progesterin receptor-immunoreactivity is not as sensitive as the diaminobenzidine technique. Rather they should be taken as evidence that some neurons which respond genomically to vaginal-cervical stimulation also contain progesterin receptors, and therefore are likely to respond to progesterone.

These findings agree with and extend the previous report that VCS increases Fos expression within estrogen receptor-containing neurons (37). It was reported that 63% of the VCS-induced Fos-IR neurons in the MPOA contained estrogen receptor-immunoreactivity (ER-IR) (37). In this study, we found that 31% of the VCS-induced Fos-IR neurons in the MPOA contain PR-IR. As estradiol-induced progesterin receptors are only seen within estrogen-receptor containing cells (20), those PR-IR neurons expressing VCS-induced Fos-IR are likely to be a subpopulation of estrogen receptor-containing neurons. Therefore, the number of PR-IR neurons is fewer than the number of ER-IR neurons. In the VMH, a greater percentage of Fos-IR neurons that contain PR-IR were observed than Fos-IR neurons that contain ER-IR. While, 24% of the VCS-induced Fos-IR neurons contain ER-IR (37), we now report that 48–54% of the VCS-induced Fos-IR neurons contain PR-IR. The difference in the percentages of colocalization between VCS-induced Fos-IR neurons with ER-IR or PR-IR in the VMH may be related to differences in the intensity of the immunofluorescence between the technique used for immunostaining of PR-IR and ER-IR. In addition, subtle differences in the intensity of Fos-IR between the two procedures might also effect the percentage of colocalization with steroid receptors.

One possible pathway by which VCS could increase Fos-IR within progesterin receptor-containing neurons is by modulating catecholamine transmission. Noradrenergic neurons project to areas in which VCS increases Fos-IR (31, 32, 37, 39–41, 53) and to areas that contain progesterin receptors. Noradrenergic transmission in the hypothalamus, which is increased during mating stimulation (54), has been shown to increase Fos expression in rat brain (55–57). In addition, electrical stimulation of the A1 and A2 region increases norepinephrine release in the preoptic area (58). The primary source of noradrenergic projections to the hypothalamus comes from the A1 and A2 cell groups (53, 59). In addition, disruption of the noradrenergic system results in reduced pseudopregnancy following VCS (60, 61). Therefore it is possible that VCS activates the noradrenergic system which leads directly or indirectly to increased Fos expression within PR-IR containing neurons. As the noradrenergic system influences steroid receptor concentration (45–47), and norepinephrine is

released during VCS (54), it is possible that VCS may regulate the concentration of progesterin receptors. That is to say, VCS may increase Fos expression, and this may then increase or decrease the expression of progesterin receptors. Although treatment with estradiol and progesterone decreases Fos expression in the VMH (39), it is not known if estradiol treatment alone alters VCS-induced Fos expression. It is possible that estrogen increases VCS-induced Fos expression and subsequent injection of progesterone decreases VCS-induced Fos expression. This is supported by the finding that estradiol increases norepinephrine-induced cAMP and subsequent treatment with progesterone decreases norepinephrine-induced cAMP in hypothalamic tissue slices (13, 62–64). The noradrenergic system is also important in modulating GnRH activity (65), and reproductive behaviors (66). Alternatively, VCS may increase Fos-IR within PR-IR neurons via a dopaminergic pathway. Recently, it was reported that mating stimuli increase dopamine release in the nucleus accumbens (67, 68) and in the VMH (54). In addition, infusion of the dopamine (D₁)-receptor agonist, SKF 38393, into the third ventricle of estradiol-primed rats increases Fos expression in areas in which VCS induces Fos-IR (J. M. Meredith, A. P. Auger, and J. D. Blaustein, unpublished observation).

In summary, our findings suggest that VCS increases Fos expression within PR-IR neurons in localized regions of female rat brain. Thus, some neurons are capable of integrating mechanostimulation provided by the male and information relating to serum progesterone levels. It is possible that VCS increases Fos expression within these areas via a catecholaminergic pathway (54, 68). VCS-induced Fos protein may then regulate the expression of other gene products within PR-IR neurons (42, 43) that are involved in reproductive behaviors.

Materials and methods

Animals

Female Sprague-Dawley rats (200–250 g) obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA, USA) were group-housed for one week in a 14:10 light:dark cycle. All rats were then ovariectomized under methohexital sodium anesthesia (52 mg/kg body weight, Brevital, Eli Lilly and Co., Indianapolis, IN, USA) prior to experiment. One week following surgery, all rats were injected subcutaneously with 10 µg of estradiol benzoate (dissolved in 0.2 ml sesame oil) followed 48 h later by either VCS (VCS+; n=8) or control perineal stimulation (VCS-; n=7). Manual stimulation of the vagina and cervix was performed, as described previously (37–39), during the dark phase of the illumination cycle. Stimulation was administered with a 1 cc plastic syringe plunger attached to a force gauge (FDN5, Wagner Instruments, Greenwich, CT, USA) in two 5 min sessions separated by a 3 min interval. Each 5 min session consisted of alternating 10 s of stimulation followed by 10 s of no stimulation. VCS+ animals received 400 g of force on the vagina and cervix, while VCS- control animals received 100 g of force applied to the perineum, as was done previously (37–39). One hour after the final stimulation, the rats were perfused. In an earlier experiment, this manual stimulation procedure resulted in an increase of Fos-IR cell number comparable to the increase in Fos-IR cell number observed following mating stimulation provided by a male rat (38).

Perfusion

Animals were anesthetized with sodium pentobarbital (89 mg/kg) and chloral hydrate (425 mg/kg). The heart was then exposed and the left ventricle was injected with 5000 units of sodium heparin dissolved in 1 ml of saline (0.15 M). The right atrium and the left ventricle were incised prior to insertion of a cannula, through the incision of the left ventricle, into the aorta. Seventy-five ml of saline preceded the flow of 250 ml of fixative (2% acrolein in 0.1 M sodium phosphate buffer; pH 7.2) through

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