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Mating activates androgen receptor-containing neurons in chemosensory pathways of the male Syrian hamster brain *

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Fos-immunoreactivity is induced during mating in the male Syrian hamster in limbic areas that relay chemosensory information and contain receptors for gonadal steroid hormones. The induction of Fos is an index of neuronal activation. After mating, c-*fos* expression is greatest in subnuclei of the medial amygdaloid nucleus (Me), bed nucleus of the stria terminalis (BNST), and medial preoptic area (MPOA). The present study determined if individual neurons in these activated subnuclei contain androgen receptors. We aim to understand how essential chemosensory and hormonal signals are integrated to control copulation Adult male hamsters ($n = 6$) were allowed to mate with a sexually receptive female for 30 min. They were perfused 1 h later with 4% paraformaldehyde and 40 μm frozen sections were processed for immunocytochemistry using antisera against Fos (Cambridge Research Biochemicals) and the androgen receptor (G.S. Prins). The brains of three non-mated males were also processed for Fos immunocytochemistry. Mating significantly increased the number of Fos-immunoreactive neurons within subnuclei of Me, BNST, and MPOA relative to non-mated males ($P < 0.05$). These nuclei contained abundant androgen receptors. In the corticomedial amygdala, 20–40% of Fos-immunoreactive neurons in mated hamsters expressed androgen receptors. Although few androgen receptors are found in the anteromedial and postero-intermediate subdivisions of the BNST, these areas exhibited 26% and 47% co-localization, respectively. In posteromedial BNST, which contains large numbers of steroid receptor-containing neurons, androgen receptors were identified in 48% of Fos-immunoreactive neurons. In the MPOA, 54% of Fos-immunoreactive neurons expressed the androgen receptor throughout the rostrocaudal extent of the medial preoptic nucleus (MPN). The largest percentage of co-localized Fos neurons (70%) was found in the magnocellular subdivision of MPN. These results provide the first direct evidence that androgen receptor neurons are activated by mating behavior, suggesting that such neurons may be incorporated in the neural circuitry underlying copulation. Further, they show that mating selectively and differentially activates androgen receptor cells.

INTRODUCTION

The central nervous system is a major target for steroid hormones secreted by the gonads; many aspects of behavior and neuroendocrine function are influenced by gonadal steroids, and the receptors for androgens and estrogens are widely but selectively distributed in the brain and spinal cord[9,43,44,46,49]. Mating behavior in the male Syrian hamster, as in many species (reviewed in ref. 3), requires the continued presence of testicular steroids. Copulation gradually declines following castration or testicular regression induced by short daylengths[33]. However, it is difficult to identify which groups of steroid receptor-containing neurons mediate the effects of testicular steroids on mating behavior due to the broad distribution of these receptors within the brain. To examine this question, the present study used the pattern of Fos immunoreactivity to identify populations of steroid receptor-containing neurons in chemosensory pathways that are activated during mating in the male Syrian hamster.

The medial preoptic area (MPOA) is critical for male mating behavior in a wide variety of species (see ref. 40 for review). Likewise, this area contains abundant steroid receptors. In the male hamster, MPOA lesions eliminate mating[37], and central implants of testosterone into the MPOA restore sexual behavior in castrated males[27]. However, the MPOA is not solely

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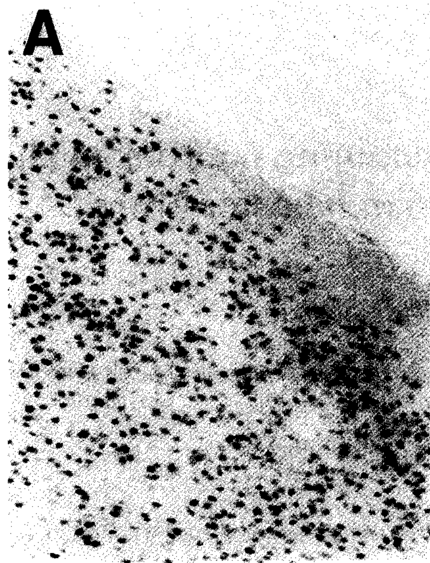
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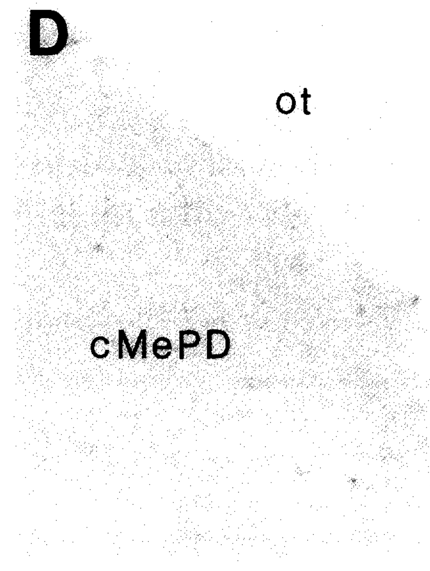
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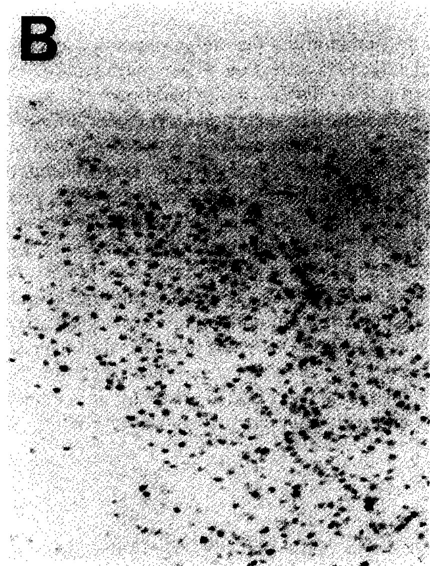
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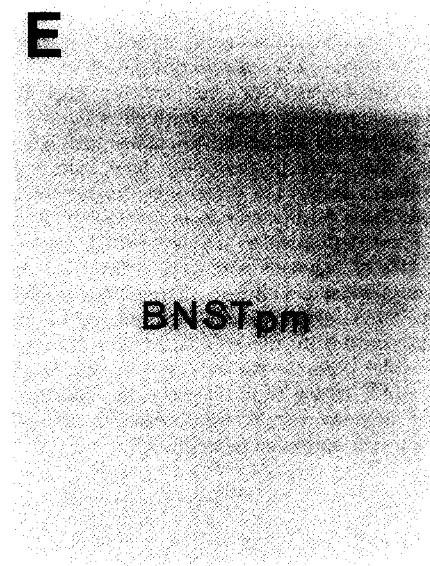
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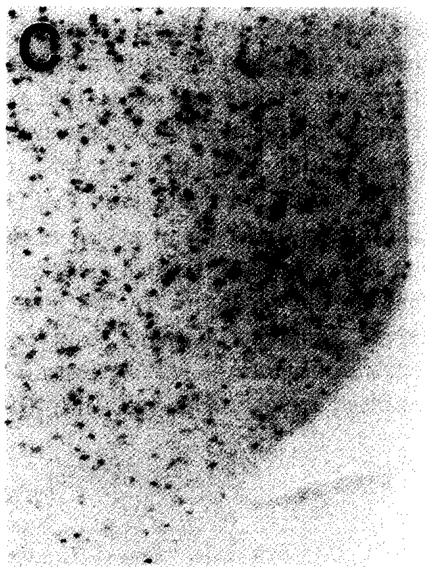
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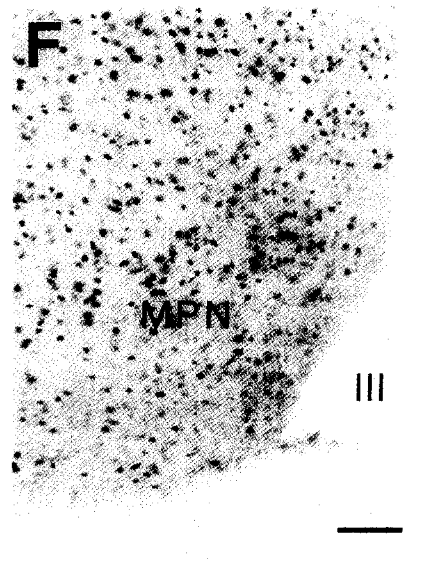
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responsible for copulation in this species; the male Syrian hamster requires both chemosensory and hormonal stimuli. Whereas steroid hormones provide a long-term permissive signal for sexual behavior[33], chemosensory input is required acutely for copulation[34]. The neural pathways for transmission of chemosensory information from the olfactory and vomeronasal systems to the MPOA have been described in detail (see ref. 40 for review). Briefly, chemosensory input via the main and accessory olfactory bulbs is transmitted along the lateral olfactory tract to the medial amygdaloid nucleus (Me). The MPOA receives projections directly from Me, as well as indirectly through the bed nucleus of the stria terminalis (BNST). Like castration, blocking the relay of chemosensory signals by lesions along this pathway eliminates mating behavior[25,26,37].

Because the MPOA, BNST, and Me transmit chemosensory information critical to the expression of sexual behavior, and because these nuclei contain large numbers of neurons that contain receptors for gonadal steroids[12,24,49], we hypothesized that androgen receptor-containing neurons in MPOA, BNST, and Me would be activated during mating. Induction of Fos, a protein product of immediate-early gene activation[19,31,41], was used as a marker of neuronal activation in mated hamsters, and the activation of androgen receptor-containing neurons was compared after mating in different subnuclei of the amygdala, BNST, and MPOA.

MATERIALS AND METHODS

General methods

Nine adult male hamsters (*Mesocricetus auratus*) weighing between 90 and 112 g were purchased from Charles River Laboratories. They were housed in groups of 3–6 per cage under a long day photoperiod (14 h light/day). Food and water were provided ad libitum.

Six males were allowed to mate with a receptive female for 0.5 h, and were perfused 1 h later. Mating tests were conducted during the first 4 h of the dark phase under dim light. During the 30 min mating test, all males displayed mounts, intromissions, and at least two ejaculations. Stimulus females were ovariectomized via dorsal incision under sodium pentobarbital anesthesia (65 mg/kg), and implanted s.c. with a Silastic capsule (id, 1.98 mm; od, 3.18 mm; Dow Corning, MI) containing a 4-mm packed column of estradiol-17B (Sigma Chemical Co., St. Louis, MO) and sealed with Silastic Adhesive A (Dow Corning). To induce lordosis, each female received 350 μg progesterone (2.5 mg/ml in sesame oil; Sigma) i.p. 4 h before the first mating test. To verify basal *c-fos* expression, three additional males were perfused without prior contact with a female. To control for non-specific effects of handling, these males were placed for 30 min in an empty cage 1 h before perfusion.

For perfusion, hamsters were deeply anesthetized with sodium pentobarbital (130 mg/kg), and perfused through the aorta with 150 ml of 0.1 M sodium phosphate-buffered saline containing 0.1% sodium nitrite for vasodilation, followed by 250 ml of 0.1 M sodium phosphate buffer (PB) containing 4% paraformaldehyde. Brains were removed and post-fixed for 1 h at room temperature and then cryoprotected overnight in PB with 20% sucrose at 4°C. 40-μm coronal brain sections were cut on a freezing microtome, and collected into PB with 0.1% sodium azide as a preservative. Sections were stored at 4°C until processed for immunocytochemistry.

Immunocytochemistry

Primary antisera. To demonstrate androgen receptor immunoreactivity, we used a polyclonal rabbit antibody (0.5 μg/ml in PB with 0.3% Triton X-100; gift of Dr. Gail S. Prins, University of Illinois-Chicago) directed against a synthetic peptide corresponding to the first 21 amino acids of the rat androgen receptor[6,28]. Fig. 1 illustrates representative androgen receptor immunostaining in Me, BNST, and MPOA. Details of the isolation and characterization of this antibody are described in Prins et al.[38]. Omission of primary antiserum eliminated nuclear staining (Fig. 1), as did preincubation with a 10× molar excess of the peptide antigen (Fig. 1). However, because the antibody is directed against only a fragment of the native receptor, preincubation of the primary antiserum with a synthetic peptide corresponding to a distant portion of the androgen receptor (amino acids 468–482) did not block immunostaining (Fig. 1).

Fos immunoreactivity was demonstrated using a sheep anti-Fos antibody supplied by Cambridge Research Biochemicals (Valley Stream, NY; lot #OA-11-823), diluted 1:1,000 in PB with 0.3% Triton X-100. Fig. 2 presents photomicrographs of immunostaining in Me, BNST, and MPOA of mated and control males. This antibody has been previously validated for use in hamsters[13] and rats[1]. Both Fos and androgen receptor immunoreactivity were confined to the cell nucleus.

Tissue processing. For single-label immunocytochemistry, free-floating sections were washed 3× for 5 min in buffer, and incubated in primary antiserum with 4% normal donkey serum for 48 h at 4°C. Sections were washed, and transferred to a biotinylated secondary antibody (donkey anti-rabbit IgG or donkey anti-sheep IgG, 1:200; Jackson Immunoresearch Labs) for 1 h at room temperature. Sections were again rinsed, then incubated in the avidin-biotin horseradish peroxidase (HRP) complex (Vectastain ABC Elite kit, Vector Laboratories) for 1 h. HRP was visualized using nickel chloride-enhanced 3,3′ diaminobenzidine (DAB) as the chromagen. Sections were mounted onto gelatin-coated slides, dehydrated in alcohols and xylenes, and coverslipped with Permount.

For double-label immunocytochemistry, the fluorescent markers FITC (fluoresceinisothyocyanate) and RITC (rhodamineisothyocyanate) were used. Fig. 3 presents photomicrographs of neurons immunostained with FITC to localize Fos immunoreactivity and with RITC for androgen receptors. For this procedure, every 4th section was incubated in a mixture of both primary antibodies, rinsed, and exposed simultaneously to donkey anti-rabbit IgG coupled to RITC (1:50) for visualization of androgen receptors and donkey anti-sheep IgG conjugated to FITC (1:50) for detection of Fos-immunoreactive neurons. To enhance the fluorescent immunostaining, these sections were exposed to unlabelled sheep and rabbit IgG (1:1,000), and then to additional fluorescent secondary antibodies. Sections were mounted onto slides and coverslipped with glycerol-phosphate buffer

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Fig. 1. Left: photomicrographs of androgen receptor-immunoreactive neurons in a representative male hamster at the level of the posterodorsal subdivision of the medial amygdaloid nucleus (top), posteromedial BNST (middle), and medial preoptic nucleus (bottom). Right: omission of the primary antiserum prevented immunostaining (top), as did preincubation with a 10× molar excess of the peptide antigen (amino acids 1–21, middle). However, preincubation of the primary antiserum with a synthetic peptide corresponding to amino acids 468–482 did not eliminate immunostaining (bottom). Bar = 100 μm.

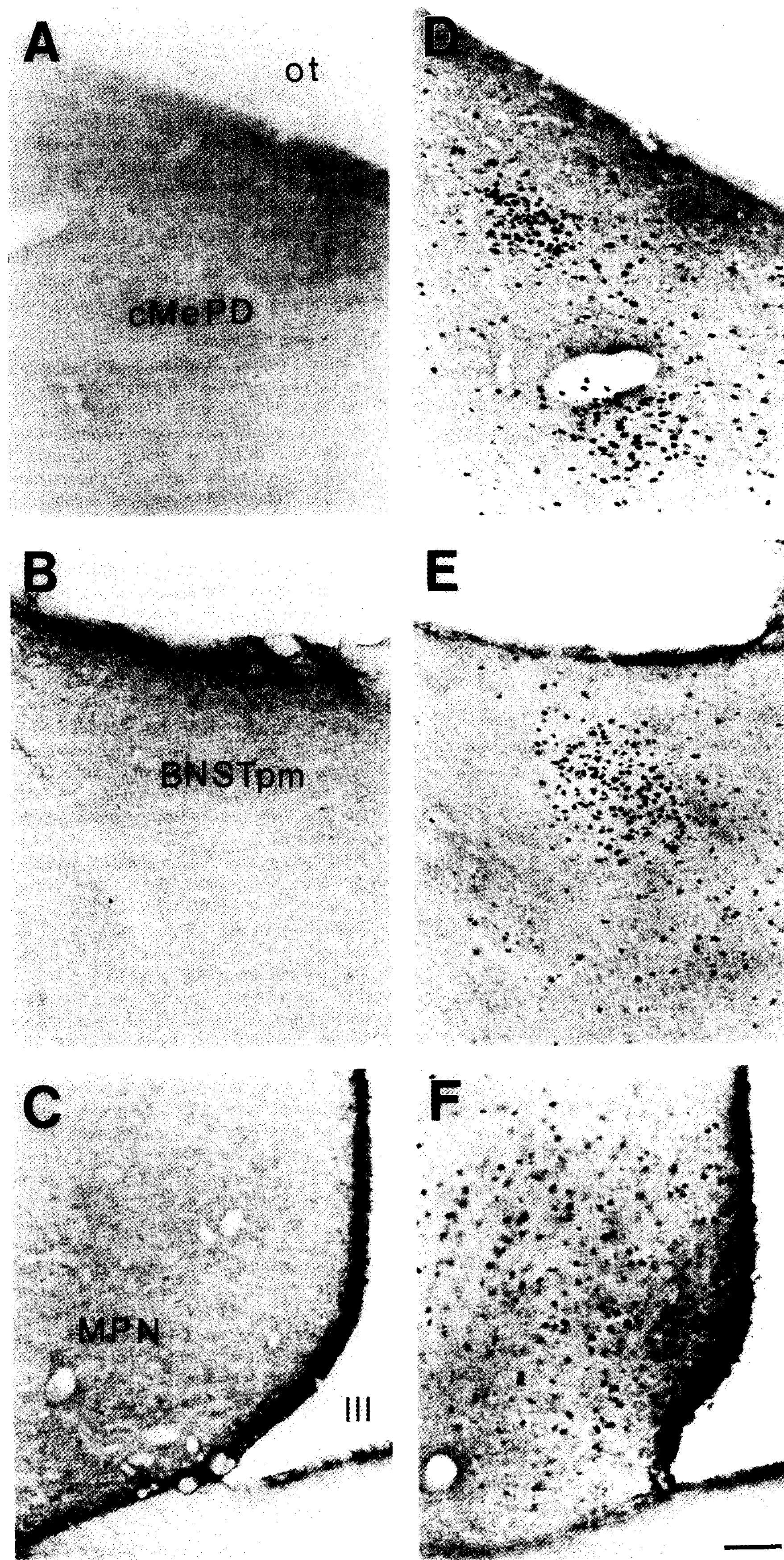

Fig. 2. Photomicrographs of Fos-immunoreactive neurons in a representative control male (left) and in a male hamster allowed to mate for 30 min (right) at the level of the posterodorsal subdivision of the medial amygdaloid nucleus (top), posteromedial BNST (middle), and medial preoptic nucleus (bottom). Bar = 100 μm.

containing phenylenediamine for preservation of FITC immunofluoresence[35]. Fluorescent staining was visualized using a Leitz Orthoplan microscope, using an I2 filter (495 nm blue light excitation) inducing green-blue emission from FITC, and an N2 filter (525 nm green light excitation) inducing red emission from RITC.

Data analysis

For single-labelled Fos and androgen receptor neurons, immunostained nuclei were plotted through a drawing tube attached to a Leitz Dialux microscope onto a series of standard sections through Me, BNST, and MPOA using cresyl violet-stained sections as a guide. For androgen receptor/Fos co-localization, we verified that the pattern of immunostaining using fluorescent markers resembled that of single-labelled sections stained with DAB. To estimate the number of Fos-immunoreactive neurons containing androgen receptors in 17 different subnuclei of the amygdala, BNST, MPOA, and septum, the average number of single- and double-labelled Fos-immunoreactive neurons within a 0.045 mm^2 area was determined three times within each subnucleus for every brain. The number of Fos-immunoreactive neurons in control and mated males was compared in subnuclei of the amygdala and BNST/MPOA using a two-way analysis of variance (ANOVA) followed by post-hoc comparison with the Scheffe F-test. The extent of co-localization in different subnuclei was likewise compared using ANOVA. For all analyses, $P < 0.05$ was considered significant.

RESULTS

Androgen receptor immunoreactivity

Fig. 1 presents photomicrographs of androgen receptor immunoreactivity in Me, BNST, and MPOA; Figs. 4 and 5 illustrate the distribution of androgen

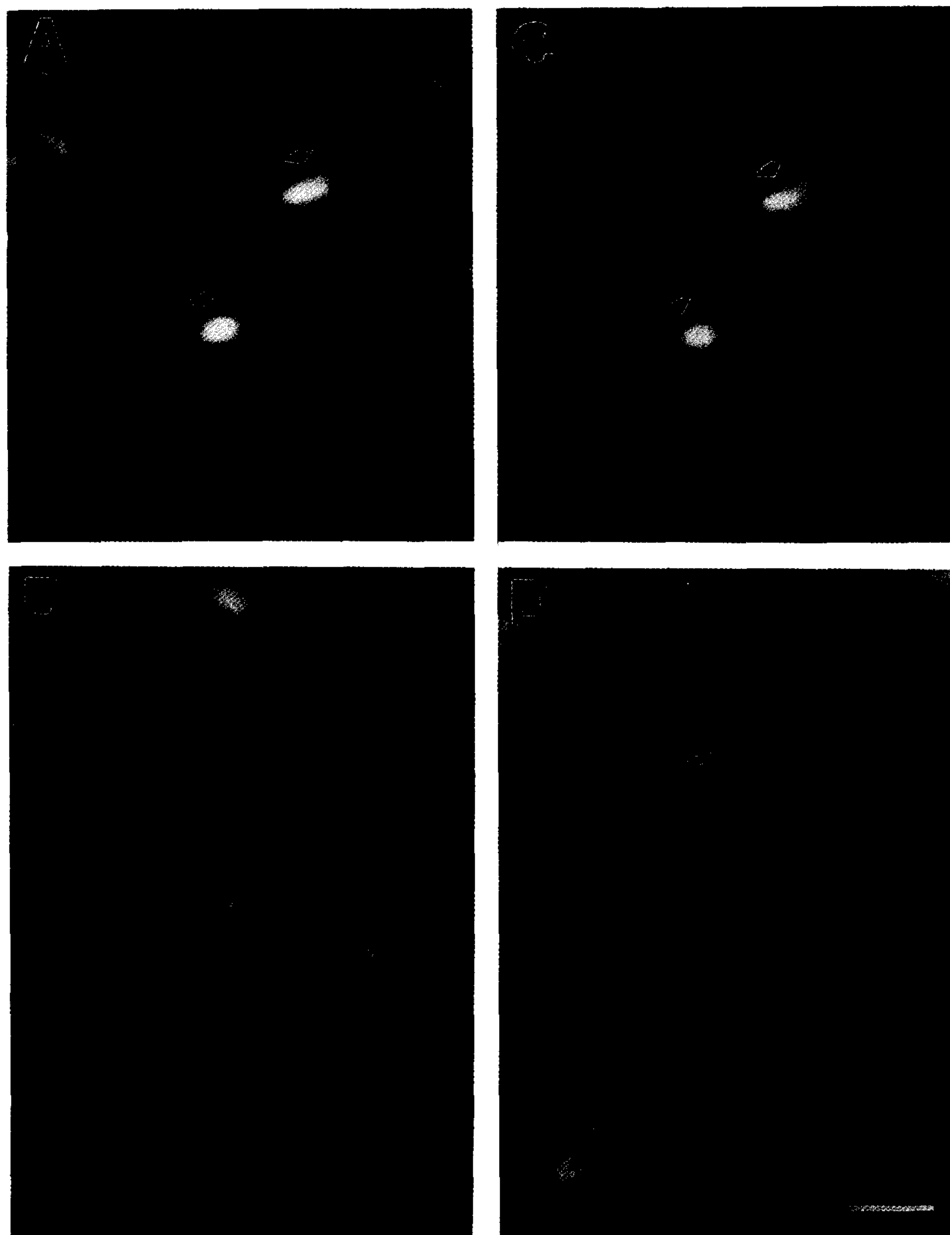

Fig. 3. Photomicrographs showing RITC-fluorescence of androgen receptor immunoreactivity (left) and FITC-fluorescence of Fos immunoreactivity (right) in the same section of the BNST from a representative male hamster. Arrows indicate neurons immunoreactive for both Fos and androgen receptors, while the arrowheads identify neurons labelled for only a single antigen. Bar = 20 μm.

receptors in these areas. The location of androgen receptors in these three regions, as well as in other areas of the brain (data not shown) agreed well with previous reports of androgen receptor distribution in the hamster brain determined using steroid autoradiography[12,49]. In general, large numbers of steroid receptors were present in Me, BNST, and MPOA, as well as in the ventrolateral septum (LSv), and the ventromedial and arcuate nuclei of the hypothalamus. However, within all of these nuclear areas, androgen receptor-containing neurons were more abundant using immunocytochemistry as compared with earlier studies using steroid autoradiography. Mating did not alter the anatomical distribution of androgen receptor immunoreactivity.

In the amygdala (Fig. 4), the majority of androgen receptors were confined to Me, the rostral part of the posteromedial cortical nucleus (PMCo, Fig. 4E), and amygdalohippocampal area (AHA, Fig. 4F). Few androgen receptors were present in other amygdaloid nuclei, including the central (Ce), lateral (L), basolateral (BL), basomedial (BM), and anterior and posterolateral cortical nuclei (ACo and PLCo). Within Me, moderate numbers of androgen receptors were found in the anterior subdivision of Me (MeA, Fig. 4A,B), but receptors were heavily concentrated in the posterior subdivision (MeP, Fig. 4D–F). This distribution in Me is equivalent to that previously described for androgen and estrogen receptors in the hamster using steroid autoradiography[12,19,49].

In BNST and MPOA (Fig. 5), androgen receptors were concentrated medially in the medial preoptic nucleus (MPN, Fig. 5C) and posteromedial BNST (BNSTpm, Fig. 5B–F), regions that receive substantial

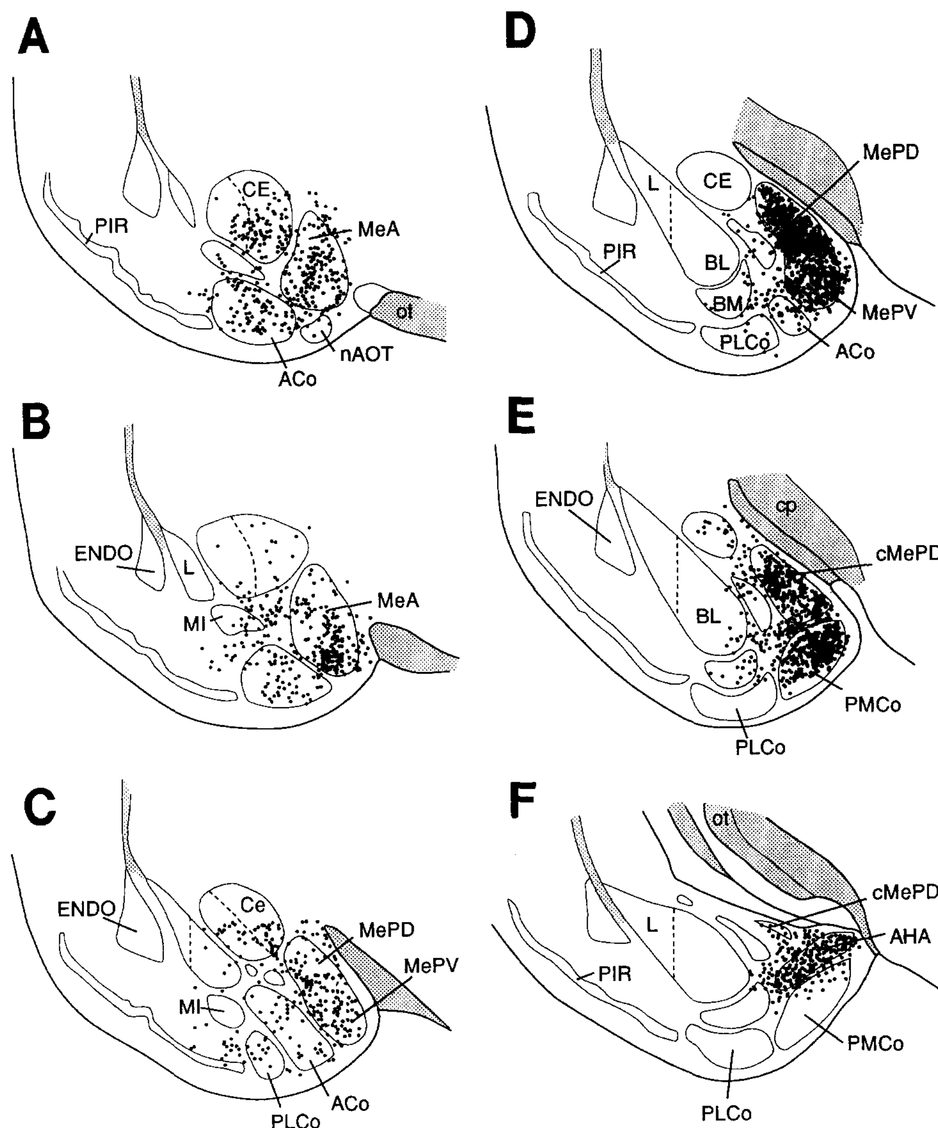

Fig. 4. Distribution of androgen receptor-immunoreactive neurons (dots) in coronal brain sections through the amygdala from rostral (A) to caudal (F) in a representative male Syrian hamster.

projections from MeP, PMCo, and AHA[18,30]. Receptors for androgens were less abundant laterally in the postero-intermediate BNST (BNSTpi) and MPOA, including the magnocellular MPN (MPNmag, Fig. 5D), areas that receive projections from MeA[18]. At the most rostral level (Fig. 5A), few androgen receptor-immunoreactive nuclei were present in the anterior subdivisions of BNST (BNSTam and BNSTal), although the receptors were abundant in the lateral septum, dorsal to the BNST. Caudally, at the transition to the hypothalamus (Fig. 5F), the majority of androgen receptors surrounded the third ventricle.

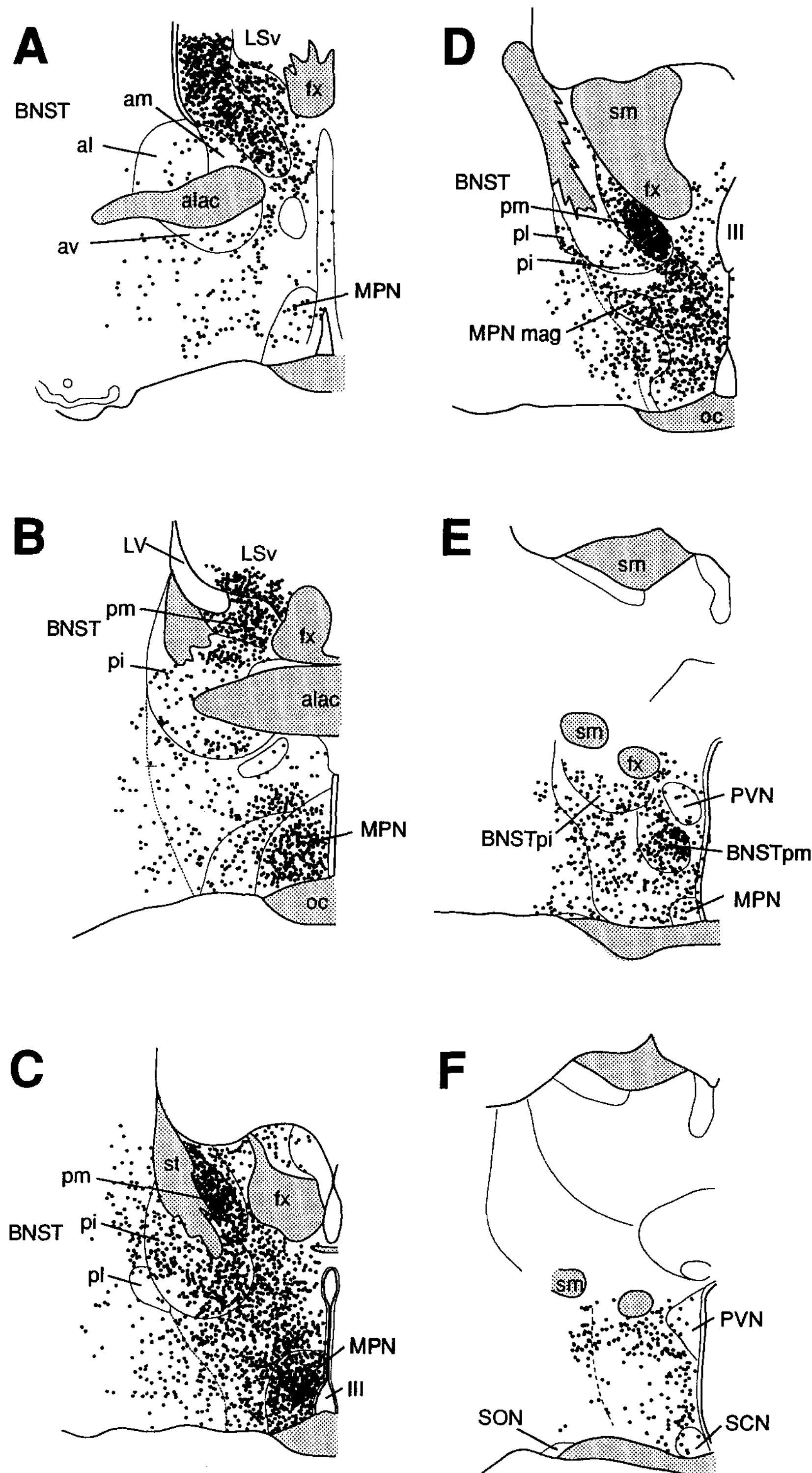

Fig. 5. Distribution of androgen receptor-immunoreactive neurons (dots) in coronal brain sections through the bed nucleus of the stria terminalis and medial preoptic area from rostral (A) to caudal (F) in a representative male Syrian hamster.

c-fos *induction during mating*

Fig. 2 presents photomicrographs of Fos immunostaining from a representative unstimulated and mated hamster in the caudal portion of posterodorsal Me (cMePD), BNSTpm, and MPN. Figs. 6 and 7 illustrate the pattern of Fos immunoreactivity in the amygdala, BNST, and MPOA in unstimulated and mated hamsters; Fig. 8 compares the number of Fos-immunoreactive neurons in subdivisions of these three nuclei. With the Fos antiserum from Cambridge Research Biochemicals, basal c-*fos* expression in unstimulated males was low (see Figs. 2, 6, 7 (left) and 8); no consistent pattern of c-*fos* expression was observed in Me, BNST or MPOA, or in other parts of the brain. However, as described for the rat[4,39] and for the hamster by Kollack and Newman [23], mating dramatically increased the number of Fos-immunoreactive nuclei in the amygdala, BNST, and MPOA (Figs. 2, 6, 7 (right) and 8).

Fos immunoreactivity was increased in mated hamsters as compared to unstimulated males throughout the rostro-caudal extent of the corticomedial amygdala ($P < 0.05$; Figs. 6 and 8). Rostrally, c-*fos* expression after mating increased 3–5 times above basal values in ACo and MeA. At more caudal levels, mating induced a 5–12-fold increase in Fos-immunoreactive neurons in MeP and PMCo, regions that contain large numbers of androgen receptors. In particular, we observed two small clusters of Fos-immunoreactive nuclei in the caudal posterodorsal subdivision of Me (Fig. 2), as previously described by Kollack and Newman[23]. Mating also increased c-*fos* expression in PLCo and AHA ($P < 0.05$), but had no discernible effect on c-*fos* expression in other regions of the amygdala, including Ce, L, BL, and BM.

Mating also significantly increased Fos immunoreactivity in BNST and MPOA ($P < 0.05$), as illustrated in

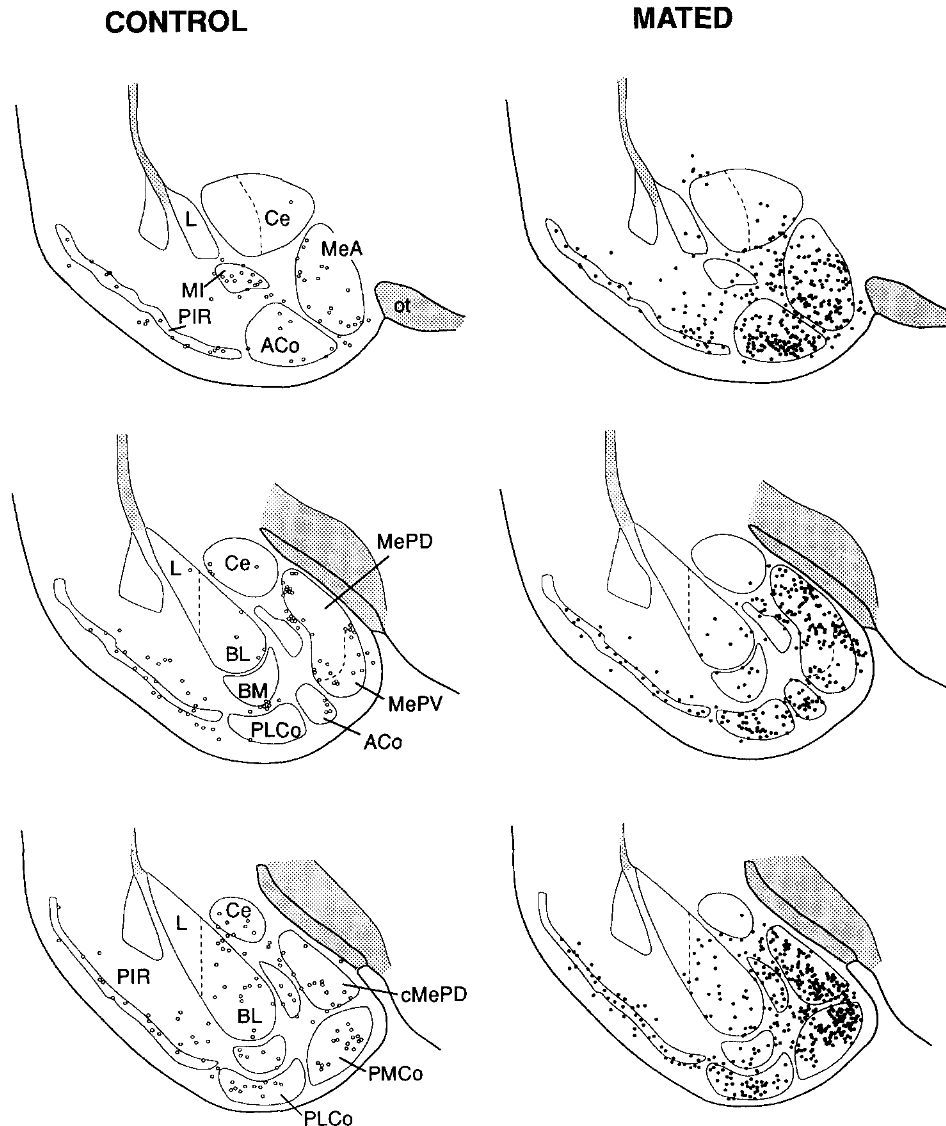

Fig. 6. Distribution of Fos-immunoreactive neurons (dots) in coronal brain sections through the amygdala from rostral to caudal in a representative control male (left) and in a male hamster allowed to mate for 30 min (right).

Fig. 7. In the BNST, the greatest increase in c-*fos* expression was observed in BNSTpm, but significant increases in Fos immunoreactivity were also noted in the anteromedial, anteroventral, and postero-intermediate subdivisions. Copulation also caused a modest increase in c-Fos expression in LSv ($P < 0.05$), immediately dorsal to BNSTam. In the MPOA, mating induced a significant increase in c-*fos* expression in both rostral and caudal MPN, areas that contain large numbers of androgen receptors. A striking increase in Fos immunoreactivity also occured in the MPNmag (Fig. 7, bottom), as reported previously by Kollack and Newman[23].

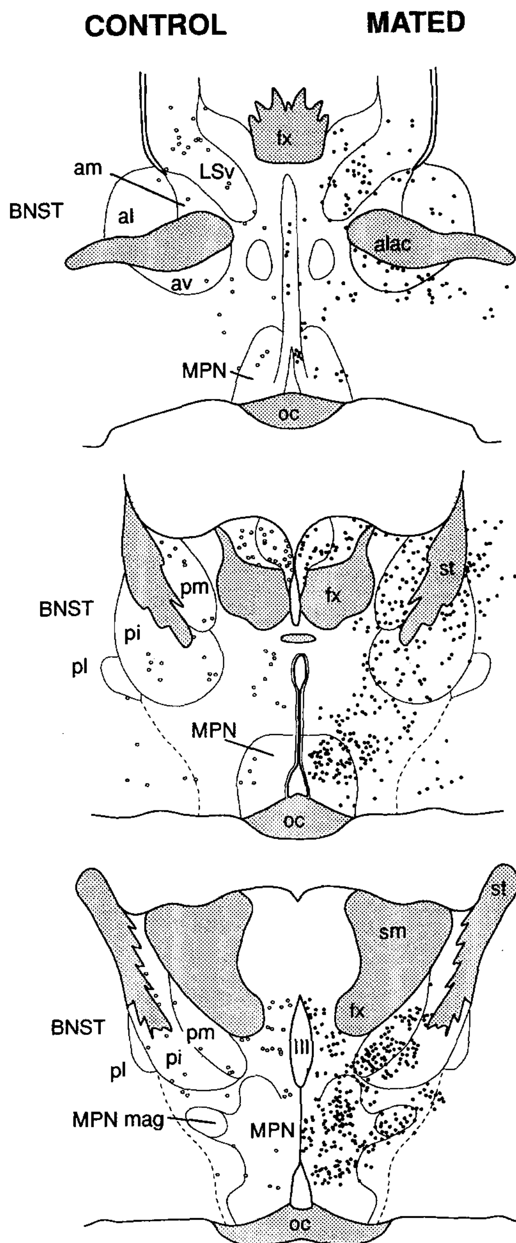

Fig. 7. Distribution of Fos-immunoreactive neurons (dots) in coronal brain sections through the BNST and MPOA from rostral to caudal in a representative control male (left) and in a male hamster allowed to mate for 30 min (right).

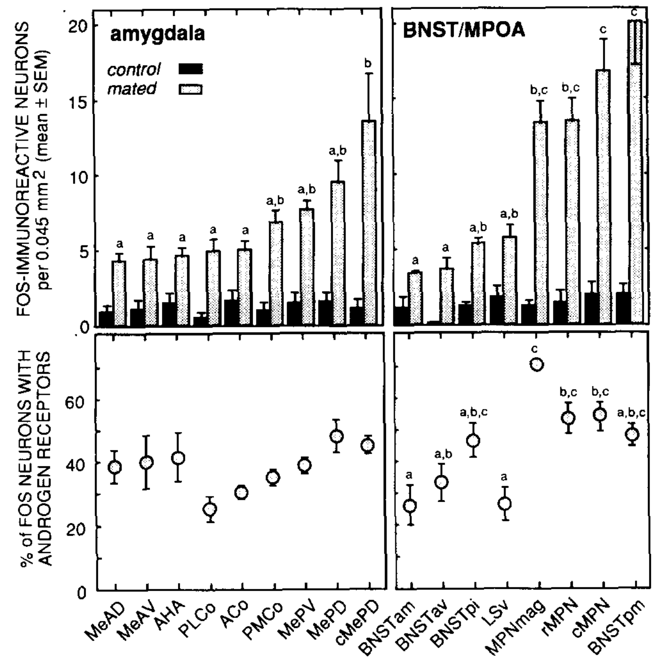

Fig. 8. Top: mean (±S.E.M.) number of Fos-immunoreactive neurons in control ($n = 3$, dark bars) and mated ($n = 5$, light bars) hamsters through subdivisions of the amygdala (left) and BNST and MPOA (right). Bottom: percentage of Fos-immunoreactive neurons that contain androgen receptors in male Syrian hamsters allowed to mate for 30 min.

Co-localization of Fos and androgen receptors

Fig. 3 presents photomicrographs of neurons in BNST that contain both androgen receptors and Fos; Fig. 8 illustrates the percentage of Fos-immunoreactive neurons that contained androgen receptors in subdivisions of the amygdala, BNST, and MPOA. In the amygdala, 25–48% of Fos-immunoreactive neurons were also immunolabelled for androgen receptors. Despite the differences in mating-induced c-*fos* expression and numbers of androgen receptor-positive neurons, there were no significant differences in the extent of co-localization in subnuclei of the amygdala ($P > 0.05$). However, the percentage of Fos neurons containing androgen receptors varied in different subdivisions of the BNST and MPOA ($P < 0.05$). In the anterior portion of the BNST, which contains relatively few Fos-immunoreactive or androgen receptor-containing neurons, only 26–33% of Fos neurons contained androgen receptors. By contrast, in the posteromedial and postero-intermediate BNST, nearly half of the Fos-immunoreactive neurons co-localized with androgen receptors. Likewise, 53–54% of Fos neurons in rostral and caudal MPN were androgen receptor-positive. However, the greatest degree of co-localization occurred in MPNmag. In this region, 70% of Fos-im-

munoreactive neurons also contained androgen receptors.

DISCUSSION

The present study demonstrates that specific populations of androgen receptor-containing neurons are activated during mating in the male Syrian hamster. Using the induction of c-*fos* as an index of neuronal activity[19,31,41], copulation activates androgen receptor-immunoreactive neurons in the amygdala, particularly in the medial amygdaloid nucleus (Me), in the bed nucleus of the stria terminalis (BNST), and in the medial preoptic area (MPOA). Although it has long been recognized that steroid receptor-containing neurons are located in brain regions controlling sexual behavior, this study demonstrates that such neurons are stimulated during mating. The results of this study suggest that neurons containing androgen receptors within brain nuclei that relay chemosensory information to control sexual behavior may function in the control of copulation in this species.

Androgen receptor immunoreactivity

Previous studies in the hamster have identified androgen receptors using autoradiography for tritiated testosterone[49] or dihydrotestosterone[12,49]. With this technique, abundant, heavily labelled neurons are located in Me, AHA, BNST, MPOA, LSv, and in the ventromedial and arcuate nuclei of the hypothalamus. In general, the distribution of androgen receptor-immunoreactivity described in the present study is similar to that reported using autoradiography, although greater numbers of androgen receptor-positive cells are detected with immunocytochemistry. Autoradiography may be a relatively less sensitive technique than immunocytochemistry because it relies on the binding of labelled hormone to its receptor, and the subsequent deposition of silver grains on a photographic emulsion in quantities sufficient to enable unequivocal signal detection. Thus, we suggest that immunocytochemistry reveals additional neurons containing low levels of the androgen receptor that have not been detected by autoradiography.

Identification of the DNA sequence for the androgen receptor[6,28] permitted the development of antibodies for immunocytochemical localization of androgen receptors in the brain and other tissues. Because it is still not feasible to purify the native receptor in sufficient quantities for innoculation, most androgen receptor antibodies have been generated with a synthetic peptide corresponding to a portion of the protein sequence. The antibody used in the present study is directed against the first 21 amino acids of the receptor protein. It appears to be specific for the androgen receptor, as evidenced by blocking studies. Furthermore, comparison of the amino acid sequences of the androgen and estrogen receptors[6,22,28] suggests that this antibody should not cross-react with the estrogen receptor. Recent studies with other similar antibodies have described the distribution of androgen receptors in the brain of the rat[42,47], monkey[8], and quail[2]. The pattern of androgen receptor immunoreactivity and the number of immunoreactive neurons we have described in the hamster brain agrees well with that reported for other mammals. Furthermore, androgen receptor immunoreactivity is similar to that of the estrogen receptor. Both receptor proteins are located predominantly within the cell nucleus, and the distribution of labelled neurons overlaps considerably[7,15,42,47]. Using a combination of immunocytochemistry and autoradiography, androgen and estrogen receptors have been localized to the same neurons in the canary brain[16]. Whether androgen and estrogen receptors co-localize in the mammalian brain has not been determined.

Fos immunoreactivity

In recent years, the expression of c-*fos*, one of the family of immediate early genes (see ref. 10), has been used as a marker for activation of individual neurons following stimulation[19,31,40]. Fos protein can be detected immunocytochemically in cell nuclei in response to variety of neural (see ref. 32) and hormonally mediated signals[5,20], including mating in the male hamster[23] and rat [4,21,39]. The pattern of c-*fos* induction following male hamster sexual behavior in the present study resembled that described in a recent report from our laboratory[23]. These two studies used different Fos antisera, which could account for the differences in basal c-*fos* expression in control males. However, the location of Fos-immunoreactive nuclei in Me, BNST, and MPOA, including the activation of two small cell groups in caudal MePD, was strikingly similar.

Previously, the functional importance of different brain areas was inferred from lesion studies; expression of c-*fos* permits identification of individual activated neurons, which can then be further characterized in terms of connections, transmitter production, or receptors. Lesions centered in BNSTpm and MPN, regions that contain the largest number of mating-induced Fos-immunoreactive neurons, severely impair chemoinvestigation and mating behavior[37]. This concordance between c-*fos* induction and behavioral deficits induced by selective brain lesions supports the use of Fos as a tool to identify functionally relevant neural pathways.

However, in the amygdala, there is some disparity between areas of marked *c-fos* induction and lesions that reduce mating. Although mating induces *c-fos* throughout Me, the greatest concentration of Fos-immunoreactive neurons is in MeP. By contrast, lesion studies suggest that the anterior subdivision of Me is critical for mating. Lesions of MeA eliminate copulation[25], whereas lesions of MeP reduce chemoinvestigation and lengthen the time to first ejaculation[26]. This difference illustrates one of the potential difficulties in interpreting the significance of *c-fos* activation. Induction of *c-fos* does not discriminate those neurons whose activation is essential for the expression of copulatory behavior from those activated merely as a consequence of the behavior. Thus, the large number of Fos-immunoreactive neurons in MeP may be a function of the amount and type of stimulation received, rather than the importance of that stimulation to mating behavior. In this regard, MeA contains only moderate numbers of steroid receptor-containing neurons, in contrast to the marked accumulation in MeP. Because MeP contains both a large number of androgen receptor-containing neurons and receives indirect olfactory and vomeronasal input via projections from MeA[18,25], neurons in this region have the potential to receive substantial stimulation from chemosensory and hormonal signals during mating. Thus, the dramatic activation of *c-fos* in MeP may reflect the presence of multiple stimulatory inputs to this area rather than the functional importance of such stimulation.

Co-localization of Fos and androgen receptors

Results of the present study demonstrate that a sub-population of androgen receptor-containing neurons are activated during mating in the male hamster. However, mating is a complex behavior, and we do not know which specific aspects of copulation account for *c-fos* expression. Exposure to female hamster vaginal secretion alone can induce *c-fos*[14], in a pattern similar to that observed after the full copulatory sequence. Mating also increases serum testosterone concentrations[29], and gonadal steroids are known to increase Fos in the brain[5,20]. At the present time, we do not know if the activation of steroid receptor-containing neurons is a result of neural or hormonal stimulation. Certainly, the mere binding of androgen to its receptor is not sufficient to induce *c-fos*. If that were so, secretion of gonadal steroids would activate the entire complement of steroid receptor neurons in the brain. Instead, only a small fraction of androgen receptor-containing neurons in the amygdala, BNST, and MPOA contained Fos protein. This suggests that *c-fos* expression reflects a more potent activation of that neuron during copulation. It is important to note that although we have not yet determined if *c-fos* is expressed in estrogen receptor-positive neurons after mating, it is likely that the pattern of co-localization would be similar to that of androgen receptors. This is based upon the overlapping distributions of androgen and estrogen receptor neurons[9,43,44,46,49], and involvement of estrogens in mating behavior in the male[17,27,36,48].

In most subdivisions of the amygdala, BNST, and MPOA that demonstrate significant increases in Fos immunoreactivity during mating, subnuclei that contained larger numbers of androgen receptors and Fos-immunoreactive neurons also tended to show greater co-localization. Two notable exceptions warrant further consideration. Despite the striking accumulation of steroid receptors in the ventrolateral septum, relatively few Fos-immunoreactive neurons in that area contained androgen receptors. However, LSv is not considered critical for mating. Instead, it is associated with grooming behavior, aggression, and scent-marking (see ref. 45). Thus, the activation of neurons in this region may be due to some behavior that occurs during mating which is not dependent upon hormonal stimulation. In contrast to LSv, the MPNmag contains relatively fewer androgen receptor-containing neurons than adjacent medial structures, but androgen receptor-containing neurons in this region demonstrated the highest degree of activation. Lesions centered around MPNmag cause severe reductions in mounts, intromissions, and ejaculations[37], suggesting that this nucleus is important for copulation. Furthermore, central implants of testosterone into the preoptic area are sufficient to restore mounting and intromissions in castrate males[27], indicating that the gonadal steroid receptors in this region are critical for mating behavior. Nonetheless, it is unlikely that neurons in MPNmag maintain the entire complement of copulatory behaviors. The activation of androgen receptor-containing neurons in subnuclei throughout the mating behavior pathway in the male Syrian hamster suggests that copulation is not solely dependent upon a single group of steroid-responsive neurons. Instead, it seems probable that different populations of androgen receptor-containing neurons throughout the limbic system maintain steroid-dependent neural elements that contribute to the complex control of mating behavior in this species.

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