Effect of a Null Mutation of the c-fos Proto-Oncogene on Sexual Behavior of Male Mice

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ABSTRACT

Sexual behavior was observed in male mice that were homozygous for a null mutation of the c-fos proto-oncogene, as well as in heterozygous mutants and wild-type controls. The onset of mounting was slower and the subsequent mounting rate was significantly lower in homozygous mutants than in either group of controls. Even so, a similar percentage of males of each genotype achieved ejaculation, and ejaculation latencies were equivalent in these mice. Likewise, in males that intromitted, the intromission efficiency and the number of intravaginal thrusts/intromission were similar among the three genotypes. The nuclear protein product (Fos) of c-fos was visualized immunocytochemically in the brains of heterozygous male mice 1 h after they exhibited a series of mounts, with or without intromission, leading to an ejaculation. As in the male of several other rodent species, nuclear Fos immunoreactivity was augmented in neurons of limbic and midbrain regions thought to convey olfactory/vomeronasal and genital/somatosensory information, respectively, to the medial preoptic area following contact with an estrous female. One interpretation of our behavioral results is that in the absence of normal neuronal c-fos expression, sensory stimuli that impinge on the male brain during mating lose their ability to initiate a cascade of further gene transcription events that otherwise control the rate at which a male reorients towards and mounts an estrous female during an ejaculatory series. Alternatively, the c-fos null mutation may disrupt normal neural development, leading to a structural change that mediates the observed deficit in mounting capacity.

INTRODUCTION

Increased neuronal expression of the proto-oncogene c-fos occurs in several spinal as well as higher forebrain regions in vertebrates after exposure to a variety of external sensory, as well as homeostatic, stimuli [1]. Several investigators have recently used immunocytochemical methods to visualize increments in nuclear Fos immunoreactivity (FOS-IR) in neurons located in several forebrain regions of male rodents after coital interaction with an estrous female. For example, in male rats killed 1 h after a series of mounts and intromissions leading to ejaculation, FOS-IR is significantly augmented in neuronal nuclei situated in the medial preoptic area (mPOA) and in the posterodorsal preoptic nucleus (PDPN), as well as in several limbic (i.e., medial amygdala [mAMYG], bed nucleus of the stria terminalis [BNST]) and midbrain (i.e., central tegmental field [CTF]) regions that project to the mPOA [2–4]. Although some of the details differ, similar results have been obtained in the male golden hamster [5, 6] and gerbil [7]. Taken together, these findings reveal a limbic-hypothalamic circuit activated in response to the genital/somatosensory and olfactory/vomeronasal stimuli that impinge on the male rodent in the course of repeated approaches to and copulation with a female. Although the monitoring of mating-induced increments in neural FOS-IR has served as a useful anatomical tool, the results obtained thus far have shed no light on the possible physiological role of c-fos expression in neurons that respond to sexual stimulation. Fos is a nuclear transcription factor; however, there is no indication to date of specific neuronal genes whose transcription is enhanced by mating-induced c-fos expression.

R.S. JOHNSON

One way of beginning to assess the possible physiological significance of increased neuronal expression of c-fos resulting from physical contact with an estrous female would be to study the sexual behavior of an animal in which this gene has been experimentally disabled. Recently, such a disruption of c-fos expression has been accomplished in mice, by use of embryonic stem cells targeted by homologous recombination at the c-fos locus [8]. Crossing progeny of chimeric mice carrying a mutant c-fos allele has yielded live offspring that are either heterozygous or homozygous for the c-fos null mutation. The surviving homozygous mutants exhibit stunted growth of the long bones, severe osteopetrosis, and lymphopenia associated with ossification of the bone marrow spaces. In experiments 1 and 2, the masculine sexual performance of groups of adult homozygous mutant males was compared with that of groups of heterozygous and wild-type control males. In experiment 3 we monitored FOS-IR in forebrain and midbrain regions of heterozygous male mice killed 1 h after an ejaculatory series with an estrous female, to confirm that mating-induced increments in neural c-fos expression occur in mice as in other male rodents.
MATERIALS AND METHODS

Animals

F1 mice (129/SvJ × C57BL/6J), heterozygous for the c-fos null mutation, were used to produce F2 litters [8] from which males of the three different genotypes (homozygous for the c-fos null mutation, −/−; heterozygous for the c-fos null mutation, +/−; and wild-type, +/+ ) were selected for use in experiments 1 and 2 (see Table 1). It will be noted that the group of homozygous mutants available for experiment 1 was significantly older than mice included in the other two groups. In experiment 2, however, there was no significant difference in the ages of the mice tested. Homozygous mutants always weighed significantly less than mice in the two control groups (Table 1). Unisexed groups of mice were housed in microisolator cages in a room in which the lights were off between 1900 and 0500 h. Standard lab chow and water were always available; homozygous mutants were also provided with lab chow moistened with infant formula, as they lack teeth. Prior to use in these experiments, all males had on several occasions been placed overnight in a cage with a cycling female. Sexual receptivity was induced in random-bred CD-1 females (Charles River Labs., Cambridge, MA; body weight ranging from 21-30 g) with s.c. injections of estradiol benzoate (20 µg/mouse) 4 and 2 days prior to behavioral testing, followed by progesterone (500 µg/mouse) 5 h prior to the onset of testing.

Procedure

Experiment 1. Individual males were tested with an estrous female for 2 h during the latter part (between 1300 and 1700 h) of the light phase of the light/dark cycle. Behavioral tests were carried out in a room adjacent to the mouse colony, with a dim light providing illumination. Males were placed alone in microisolator cages for 15 min, whereupon an estrous female was introduced and observers recorded the following: latency to first mount (nonresponders were assigned a latency of 120 min); number of mounts with or without intromission; number of mounts/time elapsed from the first mount until ejaculation or the end of the test (mounts/min); and occurrence of ejaculation, which was confirmed by inspection of the female’s vagina for the presence of a seminal plug. In the absence of mounting behavior by a particular male, a fresh estrous female was presented every 30 min. After completion of these observations, each male was left overnight with an estrous female, and the presence or absence of a seminal plug in the female’s vagina was noted the next morning.

Experiment 2. Males that ejaculated in experiment 1 (i.e., 3 +/+ , 1 +/− , and 4 −/− males), plus additional males of each genotype that had on at least one occasion deposited a seminal plug during a night with an estrous female, served as subjects. Tests were conducted during the first 4 h of the dark phase of the day/night cycle (i.e., between 1900 and 2300 h), and a single estrous female was used with each male for the duration of the test. In addition to the behavioral variables recorded in experiment 1, we noted the following: latency to the male’s first investigation of the female’s anogenital region; percentage of mounts that led to penile intromission (intromission efficiency); time elapsed between the first mount and ejaculation (ejaculation latency); number of intravaginal penile thrusts per intromission. Males in this experiment received 1–3 tests. Data from those males observed in 2–3 tests (i.e., 14% of +/+ , 50% of +/−, and 80% of −/− males, respectively) were averaged, and these values together with values from males receiving a single test were used in the calculation of overall group means. In experiments 1 and 2, statistical comparisons were made by use of the Kruskal-Wallis nonparametric one-way ANOVA.

Experiment 3. Four heterozygous males were placed individually into cages at the beginning of the dark phase of the day/night cycle (1900 h). An estrous female was introduced into each cage, and the males’ sexual behavior was recorded until ejaculation occurred. During this test, males displayed 58 ± 10 (mean ± SEM) mounts (with and without intromission) at a rate of 1.1 ± 0.3 responses per minute. Females were immediately removed after the male ejaculated, and males were subsequently left alone in the test cage. One hour later, mated and unmated control males (4 heterozygous and 2 wild-type) were anesthetized with Ketamine and Xylazine and then were perfused via the aorta with heparinized 0.1 M PBS, pH 7.2, followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed and postfixed in 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were resectioned by use of a Vibratome. Free-floating sections were moved and postfixed in 4% paraformaldehyde for 5 h prior to the use of antiserum. Rabbit antiserum against the N-terminal sequence of rat Fos amino acids 2-17 [3,9] was diluted in 0.1 M PBS/0.1% sodium azide/0.52% Triton X-100 and applied to sections for 2 h at room temperature. Sections were then washed 2–3 times with PBS and then incubated for 48 h at 4°C with an anti-FOS antiserum (at a dilution of 1:5000 in 0.1 M PBS/0.1% sodium azide/0.52% Triton X-100). The sections were then washed twice in PBS and then incubated for 48 h at 4°C with an anti-FOS antiserum (at a dilution of 1:5000 in 0.1 M PBS/0.1% sodium azide/0.52% Triton X-100) raised against the N-terminal sequence of rat Fos amino acids 2-17 [3,9]. This antiserum does not cross-react with Fos-related proteins [10].

### TABLE 1. Age and body weights of male mice used in experiments 1 and 2.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Genotype</th>
<th>N</th>
<th>Age (days)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>5</td>
<td>119 ± 13</td>
<td>36 ± 4</td>
</tr>
<tr>
<td></td>
<td>+/−</td>
<td>6</td>
<td>113 ± 8</td>
<td>37 ± 1</td>
</tr>
<tr>
<td></td>
<td>−/−</td>
<td>6</td>
<td>256 ± 27*</td>
<td>16 ± 1*</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>7</td>
<td>175 ± 46</td>
<td>34 ± 3</td>
</tr>
<tr>
<td></td>
<td>+/−</td>
<td>8</td>
<td>203 ± 31</td>
<td>38 ± 2</td>
</tr>
<tr>
<td></td>
<td>−/−</td>
<td>5</td>
<td>257 ± 42</td>
<td>17 ± 1*</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SEM.

*aAge at the first of these tests.

*p < 0.05; Kruskal-Wallis test comparisons.
**TABLE 2. Sexual behavior of groups of male mice tested during the light phase of the L:D cycle and then left overnight with an estrous female (experiment 1).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>+/+ (N = 5)</th>
<th>+/- (N = 6)</th>
<th>-/- (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mounts (with or without intromission):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% responding</td>
<td>80</td>
<td>67</td>
<td>17</td>
</tr>
<tr>
<td>latency (min)</td>
<td>38 ± 21</td>
<td>57 ± 22</td>
<td>105 ± 16</td>
</tr>
<tr>
<td>number/test</td>
<td>7 ± 3</td>
<td>12 ± 6</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>per minute</td>
<td>0.10 ± 0.04</td>
<td>0.13 ± 0.06</td>
<td>0.003 ± 0.003*</td>
</tr>
<tr>
<td>% Ejaculating during test</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% depositing plug overnight</td>
<td>100</td>
<td>100</td>
<td>67</td>
</tr>
</tbody>
</table>

aData are expressed as mean ± SEM.
*p = 0.05; Kruskal-Wallis test comparisons.

Brain sections were subsequently rinsed four times in PBS containing 0.02% Triton X-100 and incubated for 2 h at room temperature with biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA; diluted 1:200 in 0.1 M PBS/0.1% sodium azide/0.52% Triton X-100). After four rinses in PBS, the sections were incubated with avidin-biotin-peroxidase complex solution prepared according to the manufacturer's directions (Vector Laboratories, Elite Kit) for 1.5 h at room temperature. After four washes in 0.05 M Tris-buffered saline (TBS; pH = 7.6) and incubation with 0.025% 3,3'-diaminobenzidine, 0.2% nickel ammonium sulfate, and 0.02% H₂O₂ in TBS for 5 min, sections were rinsed three times in TBS, mounted onto gelatin-coated slides, rinsed with distilled water, dried, and coverslipped by means of Permount.

Quantification of FOS immunoreactive nuclei present in neurons of different brain regions was carried out by the same investigator. Slides were coded to conceal the identity of the mated and unmated subjects. Comparable brain regions (the side was randomly chosen) were selected for counting with the help of templates of brain sections from one mated male, previously drawn using a camera lucida. All neurons containing immunoreactive nuclei in a field viewed under the 20× objective (0.466 mm²) were drawn using a camera lucida. These FOS-IR neurons were later counted in each of five brain regions, were expressed as mean ± SEM, and were analyzed using one-tailed Mann-Whitney U tests.

Finally, in order to confirm that FOS-IR was absent in forebrain neurons of homozygous mutant mice after incubation with the primary Fos antisera used in this experiment, an additional male mouse of each genotype was transported from its home cage to the laboratory, anesthetized, and perfused; and the brain was processed for FOS-IR. Mice treated in this way normally display a robust level of FOS-IR in neurons of the primary olfactory (piriform) cortex.

**RESULTS**

**Experiment 1**

Although the percentage of males mounting tended to be lower, the latency to the first mount tended to be longer,
Fig. 1. Photomicrographs showing FOS-IR neurons in the mPOA, PDPN, and BNST (A and B); the mAMYG (C and D); and the midbrain CTF (E and F) of representative male mice killed 1 h after an ejaculatory series with an estrous female (A, C, E) or after being left alone in a test cage (B, D, F). ac, anterior commissure; ma, mAMYG; ot, optic tract; st, stria terminalis. Scale bar = 200 μm.
FIG. 2. Photomicrographs showing FOS-IR neurons in the PVN of representative male mice killed 1 h after an ejaculatory series with an estrous female (A) or after being left alone in a test cage (B). Scale bar = 200 μm.

and the number of mounts with or without intromission tended to be lower in homozygous mutant males than in heterozygous or wild-type controls, these effects did not reach statistical significance (Table 2). Mounting rates were, however, significantly lower in homozygous mutants than in the other two groups. The incidence of ejaculation was low in all three groups, perhaps because this experiment was carried out during the light phase of the day/night cycle. Indeed, a similar high proportion of males of all three genotypes successfully deposited seminal plugs in estrous females in the course of the night that followed these direct observations (Table 2).

**Experiment 2**

Homozygous mutant males tended to initiate anogenital investigation of females more slowly and subsequently displayed more investigations than males in the two control groups (Table 3); however, these differences were not statistically significant. The latency to first mount was significantly longer whereas the number of mounts per test and mounting rates were significantly lower in homozygous mutants than in the other two groups of males (Table 3). Despite these significant group differences in the display of mounting behavior, there was no significant difference in the percentage of males achieving ejaculation during at least one test, and ejaculation latencies were equivalent in these mice. Likewise, in males that intromitted, the intromission efficiency and the number of intravaginal thrusts per intromission were equivalent among the three genotypes. It is noteworthy, however, that two out of three homozygous mutant males that ejaculated did so after achieving only 1 and 3 mounts with intromission, respectively, (plus several additional mounts without intromission) whereas the nine males in the other two groups that ejaculated required at least 8 mounts with intromission.

**Experiment 3**

Heterozygous males killed 1 h after a series of mounts, with and without intromission, leading to ejaculation exhibited a striking increase in FOS-IR in the mPOA, PDPN, and BNST (Fig. 1, A and B), in the mAMYG (Fig. 1, C and D), and in the CTF (Fig. 1, E and F). In addition, ejaculation augmented FOS-IR in neurons of the rostro-lateral portion of the paraventricular nucleus (PVN; Fig. 2, A and B). Quantitative analysis showed that there were significantly more FOS-IR nuclei in the mPOA, BNST, mAMYG, CTF, and PVN of ejaculating males than in those of unpaired control mice.
FIG. 3. Photomicrographs showing FOS-IR neurons in the piriform cortex of male mice of three different genotypes: wild-type (A), heterozygous for the c-fos null mutation (B), and homozygous for the c-fos null mutation (C). FOS-IR was absent from neuronal nuclei of the homozygous mutant’s brain (C). ot, optic tract. Scale bar = 200 μm.
(Table 4). Inspection of serial fore- and midbrain sections revealed no other regions in which major increments in FOS-IR occurred after mating. A comparison of FOS-IR in neurons of the piriform cortex (Fig. 3) revealed an absence of immunoreactive cell nuclei in the homozygous mutant as compared with the heterozygous and wild-type control mice.

**DISCUSSION**

Experiment 3 showed that mating augmented neuronal FOS-IR in specific regions of the male mouse’s midbrain (e.g., CTP and diencephalon, e.g., mAMYG and BNST). Neurons in these regions are thought to convey genital/somatosensory [3] and olfactory/vomeronasal [11] information, respectively, to the mPOA, where FOS-IR was also increased. These results corroborate the results of several studies using other male rodents, including rats, hamsters, and gerbils (see Introduction), as well as a previous observation made in male mice (J. Wu, personal communication). In the present study, neural FOS-IR was compared in males that either mounted, intromitted, and ejaculated with, or were not exposed to, an estrous female. No attempt was made to assess the effects of a range of sexual stimuli in these mice. However, in a previous experiment [3] using male rats, significant increments in FOS-IR were observed in the mAMYG, BNST, and mPOA after interaction with an estrous female that involved anogenital sniffing and other body contacts, but no mounting or intromission. No increments in neural FOS-IR were observed in other groups of sexually experienced male rats that were 1) exposed to an estrous female kept inside a clear Plexiglas box with holes around the base or 2) allowed to mount estrous females whose vaginas were masked with tape. In other experiments using rats, bilateral transection of the pelvic nerves blocked mating-induced increments in FOS-IR in mAMYG, BNST, and mPOA of females, whereas in males it only partially reduced FOS-IR in these brain regions [4, 12]. Also, unilateral lesions of the olfactory peduncle significantly reduced mating-induced FOS-IR in the ipsilateral mAMYG of males, provided their penes were anesthetized with lidocaine anesthetic paste [3]. These latter two findings imply that in males, nongenital stimuli (possibly of olfactory/vomeronasal origin) normally contribute to the increased neural expression of c-fos resulting from exposure to an estrous female. Finally, in male hamsters the application of estrous females’ vaginal secretions to the nose significantly stimulated FOS-IR in the mAMYG, BNST, and mPOA [13]. These results imply that olfactory/vomeronasal stimuli derived from physical contact with an estrous female, even in the absence of genital stimulation, facilitate the expression of c-fos in these brain regions.

In experiments 1 and 2, homozygous mutant male mice, which lacked a functional c-fos gene, were slow to initiate mounting of an estrous female. Once they began mounting, homozygous mutants displayed significantly lower mounting rates than heterozygous and wild-type controls. In experiment 1, the homozygous mutant males being studied were significantly older than males in the two control groups, raising the question of whether the observed differences in mounting behavior were an artifact of this age difference. This seems unlikely insofar as a similar behavioral deficit was observed in the homozygous mutants studied in experiment 2, in which there was no significant age difference among the three groups tested.

One plausible interpretation of the present results is that neuronal c-fos expression in circuits projecting to the mPOA somehow contributes to the maintenance of sexual arousal in male rodents during a series of mounts and intromissions leading to ejaculation. To date, nobody has assessed the minimal time required for an increase in neural FOS-IR to occur in the male rodent forebrain after exposure to olfactory/vomeronasal cues emanating from an estrous female. In male rats, an increase in FOS-IR in the mAMYG and mPOA was noted approximately 30 min after the onset of a series of mounts and intromissions leading to ejaculation [3]. It is possible that future studies will show that Fos protein is detected in neuronal nuclei even more rapidly after exposure of males to an estrous female. In experiment 2, the mean mount latencies for wild-type and homozygous mutant male mice were 9 and 21 min, respectively, suggesting that intraneuronal Fos proteins play little role in promoting sexual arousal that leads to the onset of mounting. However, males in these groups typically continued to display mounting and intromission with estrous females for more than an hour prior to ejaculating (see ejaculation latencies in Table 3). This is sufficient time for maximal levels of Fos protein to be synthesized, dimerize with Jun protein, bind to nuclear AP-1 binding sites, and begin to promote the further transcription of unspecified genes encoding proteins that may facilitate masculine sexual arousal. After repeated ejaculation with an estrous female, male rats and other rodents become refractory to sexual stimulation for several days [14]. The possibility that neuronal increments in nuclear Fos protein may initiate a cascade of further gene transcription underlying sexual refractoriness has been proposed [3]. At present we have no information about the occurrence of sexual refractoriness in male mice bearing a null mutation of the c-fos gene.

The homozygous mutant male mice studied in experiments 1 and 2 had lacked functional c-fos genes in brain tissue, as well as other somatic tissues, throughout their entire lives, and not solely at the time they were tested for mating behavior. Thus we cannot rule out the possibility that the behavioral deficits observed in these males reflected this fact. For example, c-fos expression may be required for the programmed cell death that normally occurs in the nervous system, bone, and other somatic tissues during development [15, 16]. An attenuation of the normal progression of neuronal apoptosis during perinatal life may
underlie the deficits in masculine sexual behavior displayed in adulthood by mutant males. More research is needed before this possibility can be ruled out. Alternatively, an absence of c-fos expression in specific brain regions of adult male rodents may lead to the deficits in the expression of masculine sexual behavior, such as those seen in the mutant mice studied in experiments 1 and 2. In future studies, the localized infusion of antisense oligodeoxynucleotide to c-fos mRNA into brain regions that normally express c-fos after mating may be used as an alternative method [17] for assessing the contribution of Fos protein to masculine sexual arousal and performance.

In addition to limbic regions (i.e., the mAmy and BNST), which convey olfactory/vomeronasal information to the mPOA, and a midbrain region (i.e., the CTF), which may convey genital/somatosensory information to the mPOA, an intense mating-induced c-fos response was noted in the PVN of male mice following ejaculation (see Fig. 2). A similar, albeit less intense, c-fos response was previously noted in the PVN of male rats following ejaculation [3]. Insel and Witt [18] have suggested that this mating-induced c-fos expression occurs in parvicellular oxytocin neurons of the PVN. Indeed, several studies (reviewed in [19]) have implicated oxytocin in the control of penile erection in the rat. Neurochemical and immunocytochemical data from the male mouse suggest that mating promotes the immediate release and subsequent synthesis of oxytocin in PVN neurons [20]. Alternatively, it is possible that the FOS-IR induced by mating in the male mouse PVN may be localized in corticotrophin-releasing-hormone neurons, insofar as there is considerable agonistic, and thus potentially stressful, interaction during the course of mating in this species.

Recent observations (J.J.G. Brown and V.E. Papaioannou, unpublished observations) showed that two of the sexually experienced, homozygous mutant male mice used in the present studies failed to impregnate fertile females, even though the animals were housed together continuously for over 1.5 mo. Four additional mutant males failed to impregnate fertile females over 1.5–7 mo of cohabitation. The low number of mounts, with or without intromission, and the slow rate of mounting characteristic of mutant males may be factors contributing to their low fertility. This particular pattern of masculine coital behavior may provide insufficient vaginal-cervical stimulation to establish the postcoital gestational state in females [21]. Alternatively, deficiencies in spermatogenesis may underlie this observed infertility of mutant males [8]. It should be noted that Wang et al. [22] stated that their male mice bearing a c-fos null mutation were “fertile,” although no data pertaining to this claim were presented in their paper.

Despite the reticence of homozygous mutant male mice to initiate and subsequently sustain mounting and intromission behavior with estrous females, the present observations show that these animals are capable of displaying all of the behavior patterns associated with mating, including ejaculation and the deposition of a seminal plug in the female’s vagina. These observations are consistent with previous observations in male rats [3, 4], suggesting that there is no relationship between the ability to exhibit masculine sexual behavior patterns per se, and the expression of c-fos in any brain region. The present results also imply that the small stature of homozygous mutants (body weights were less than half those of males in the two control groups) cannot account for the observed deficits in the temporal patterning of their sexual behavior. When homozygous mutants exhibited mounts, they were just as likely to achieve penile intromission as males in the two control groups. Likewise, once intromission occurred, the number of intravaginal thrusts was equivalent in the three groups of mice.

The expression of sexual behavior in adult male mammals, including mice, is thought to depend on both the perinatal (“organizational”) [23, 24] and the adult (“activational”) [25, 26] actions of testosterone, or its estrogenic metabolites, in the brain. It seems unlikely that the observed deficits in mutant males’ coital performance can be explained by deficiencies in either of these steroid-dependent processes. First, while it is true that somatic development was greatly attenuated in mutant males, all indications are that steroid-dependent brain sexual differentiation was normal in these mice, since mutant males were capable of displaying all components of masculine sexual behavior. This contrasts with the striking reduction in the coital responsiveness of androgen-insensitive (Tfm/N) male mice, in which aspects of brain sexual differentiation are thought to be deficient [27]. Second, while we have no information on the circulating levels of testosterone present in adult males of the three genotypes studied here, it is clear that sufficient testosterone was present in all groups to activate sexual behavior when mice were presented with a steroid-primed estrous female during the dark phase of the day/night cycle. A previous study [28] using male mice of eight different strains found no correlation between plasma testosterone levels and behavioral measures of sexual arousal and ejaculatory performance. Thus it seems unlikely that the deficient mounting performance of homozygous mutants in the present study can be attributed to lowered testicular androgen secretion, even if this is found to occur.

Several reports [29–32] have shown that exposure of rats or hamsters to light pulses during the animal’s subjective night stimulates c-fos expression in a set of neurons of the suprachiasmatic nuclei that receive direct retinal inputs. It has been found that mice homozygous for the c-fos null mutation display circadian activity rhythms but do not entrain normally to a day/night photoperiod (R. Johnson, B. Spiegelman, V. Papaioannou, and M. Ralph, unpublished observation). This raises the possibility that the deficient coital responsiveness of homozygous mutants was simply a consequence of a general lowering of activity at the time of testing. It is noteworthy that mounting and ejaculatory performance were lower in wild-type and heterozygous
controls tested during the light (experiment 1) as opposed to the dark (experiment 2) phase of the day/night cycle. Although the absolute level of mounting behavior was lower in both studies, a comparison of homozygous mutants in experiments 1 and 2 reveals a similar profile, with somewhat higher mounting rates being displayed in tests given during the dark period. Inspection of Table 3 shows that during these latter tests homozygous mutant males apparently tended to display more anogenital investigations of the estrous females, usually in the absence of mounting, than males in the other two groups. Homozygous mutant males often displayed these bouts of anogenital investigation after they efficiently pursued estrous females that were fleeing at great speed. Also, casual observation revealed that some homozygous mutants displayed an intermittent jumping response for periods lasting up to an hour during tests given in both experiments 1 and 2. Taken together, these observations suggest that the deficient mounting performance of homozygous mutants cannot be attributed to a generalized lack of behavioral arousal during the period of testing. Instead, their deficiency seems to reflect an inadequate temporal integration of signals from the female needed to coordinate the ongoing expression of their mounting responses.

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