Demonstration of Ejaculation-Induced Neural Activity in the Male Rat Brain Using 5-HT_{1A} Agonist 8-OH-DPAT

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COOLEN, L. M., B. OLIVIER, H. J. P. W. PETERS AND J. G. VEENING. Demonstration of ejaculation-induced neural activity in the male rat brain using 5-HT_{1A} agonist 8-OH-DPAT. PHYSIOL BEHAV 62(4) 881-891, 1997.—Previous studies from our laboratory indicated the existence of ejaculation-related neural activation within the circuitry underlying mating behavior in the male rat. Clusters of Fos-immunoreactive neurons were present only following ejaculations and not after intromissions. However, it was not clear if this pattern of neural activation was specific to ejaculation or a result of summation of sexual activity preceding ejaculation. In the present study, the facilitative effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT on ejaculatory behavior was used to analyze the pattern of Fos immunoreactivity following ejaculation preceded by minimal sexual activity. Male rats treated with 8-OH-DPAT (0.4 mg/kg) achieved ejaculation after a shortened latency and low numbers of mounts and intromissions. Ejaculation-induced Fos immunoreactivity was present in clusters of neurons in the lateral part of the posterodorsal medial amygdala, in two subregions of the posteromedial bed nucleus of the stria terminalis, in the posterodorsal preoptic nucleus, and in the parvicellular part of the subparafascicular thalamic nucleus. Males that ejaculated with the first intromission and were treated with a higher dose of 8-OH-DPAT (0.8 mg/kg) exhibited similar clusters of Fos-positive neurons in all areas except the posterodorsal preoptic nucleus. The results demonstrate the existence of a specific ejaculation-related subcircuit within a larger neural circuitry involved in male sexual behavior. © 1997 Elsevier Science Inc.

Sexual behavior c-fos Serotonin Medial amygdala Medial preoptic area Subparafascicular nucleus Bed nucleus of the stria terminalis

IN recent years, immunocytochemical visualization of Fos, the protein product of the immediate early gene c-fos (31), has been used to map functional circuits underlying sexual behavior in the male and female rodent brain. Copulation increased Fos immunoreactivity (IR) in the medial preoptic area (MPOA), medial amygdala (MEA), bed nucleus of the stria terminalis (BNST), and central tegmental field in male and female rats (3,13,35,36,42), male hamsters (15,25,43), male gerbils (20), and male and female ferrets (27). These brain regions are components of a neural network underlying the regulation of sexual behavior (30). Previous studies from our laboratory have demonstrated that Fos-IR in male rats is selectively induced in distinct neuronal clusters within the limbic circuitry following ejaculation, but not after intromissions alone. These clusters are located in the posteroventral MEA (MEApd), posteroventral BNST (BNSTpm), posteroventral preoptic nucleus (PD), and parvicellular subparafascicular thalamic nucleus (SPFp) (8,9). However, since Fos-IR was studied following full mating, it could not be determined whether this pattern of Fos-IR was specifically related to ejaculation itself or instead reflected a summation of sexual activity including ejaculation but also intromissions and mounts that preceded the ejaculation.

The present study tested the hypothesis that the Fos-positive neuronal clusters present after ejaculation are related to ejaculation per se, rather than preceding sexual activity. If so, this would imply the existence of an ejaculation-specific circuit within the larger circuitry for male sexual behavior. The approach was to determine the distribution of Fos-positive neurons after mating preceded by a minimum of sexual contact. To accomplish this, male rats were injected with the serotonergic 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT) (0.4 mg/kg), which has a strong facilitatory effect on male ejaculatory behavior. 8-OH-DPAT reduces both ejaculation latency and the numbers of intromissions and mounts preceding ejaculation (1,2,16,19,22).

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Animals and Treatment

Thirty minutes later, a receptive female was introduced. Mating males were observed from the time of injection until the end of the mating test to determine the expression of copulatory behavior and the behavioral effects of 8-OH-DPAT. After testing, males were returned to their home cages. Water was available, but food was removed from all cages to prevent induction of Fos-IR as a result from 8-OH-DPAT-induced food intake (10,12). Animals were sacrificed 60 min after the end of testing.

Experiment 2

Administration of 8-OH-DPAT in Experiment 1 facilitated ejaculatory behavior (Table 1), allowing analysis of patterns of ejaculation-induced Fos-IR in males that displayed low numbers of mounts and intromissions. In Experiment 2, a higher dose of 8-OH-DPAT (0.8 mg/kg b.wt.) was used to produce males that ejaculated during the first and only intromission. Sexually experienced males were injected with 8-OH-DPAT (0.8 mg/kg b.wt., s.c.) and placed in a mating arena immediately after injection. Thirty minutes later, a receptive female was introduced. Only males ejaculating in copula with the first mount, which was thus an intromission, were selected for further study (n = 3). After the first ejaculation, these males were returned to their home cages and were sacrificed 60 min later. Similar to Experiment 1, control animals (n = 3) were injected with 8-OH-DPAT but did not interact with a receptive female. Instead they were placed alone in a clean test cage for a similar period of time as the mating animals.

Perfusion, Tissue Processing, and Immunocytochemistry

Animals were anaesthetized using sodium pentobarbital (Narcovet, 60 mg in 1 mL i.p.), injected with heparin (4 mg in 1 mL i.p.) to prevent blood clotting, and perfused transcardially with 0.1

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TABLE 1

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Scores are mean seconds (±SEM) of latencies until first mount, first intromission, and first and second ejaculations, mean seconds of the postejaculatory interval following the first ejaculation, and mean numbers of mounts and intromissions preceding the first and second ejaculations in mating males treated with saline (SAL-M) or 8-OH-DPAT (DPAT-M). Significant increases (↑) or decreases (↓) in DPAT-M compared to SAL-M males are indicated by asterisks (p < 0.05).

EXPERIMENTAL PROCEDURES

Animals and Treatment

Male Wistar rats (n = 15, 260–285 g, 2–3 months old), obtained from the local breeding facilities of the University of Nijmegen, were housed in pairs in an artificially lighted room (LD 12–12, lights off at 0600 hours) apart from females. Forty-eight hours before testing, males were housed individually. Food and water were available at all times. Female Wistar rats (200 g, 2 months old) were bilaterally ovariectomized for use as stimulus females. Sexual receptivity in these females was induced with estradiol benzoate (50 μg, dissolved in 0.1 mL of arachidis oil, s.c., subcutaneously) 48 h before testing, and progesterone (500 μg, dissolved in 0.1 mL of arachidis oil, s.c.) 4 h before testing.

Experiment 1

Four groups of sexually experienced male rats (n = 3 each) were used. Testing was performed 6 h after lights off. Animals received mating experience during four pretest mating sessions. Males were considered sexually experienced when displaying multiple ejaculations and the first ejaculation within 10 min after onset of the last two mating tests. Two groups of males were allowed to copulate with a receptive female in the mating arena (40 × 50 × 65 cm) filled with clean bedding, which was the same environment in which they gained mating experience. One group of males (DPAT-M) was injected with 0.4 mg/kg b.wt. 8-OH-DPAT (RBI, Natick, MA, USA; diluted in normal saline and injected s.c. in a volume of 0.2 mL), while males in the second mating group (SAL-M) were s.c. injected with an equal amount of saline. Immediately after injection, males were placed in the mating arena. Thirty minutes later, a receptive female was introduced. Mating was stopped after the second ejaculation (ca. 24 min in the SAL-M group and 11 min in the DPAT-M group; see Table 1). The remaining males were injected with 8-OH-DPAT (DPAT-C) or saline (SAL-C) but did not interact with a receptive female. Instead, they were placed alone in a clean test cage containing clean bedding for 40–45 min following injection. The behavior of all males was observed from the time of injection until the end of the
In Experiment 1, the number of monos and triominoes was recorded in addition to the position of the two monos and the triomino, as shown in Table 1. The number of monos and triominoes was also recorded in Experiment 2, as shown in Table 2. The data obtained in Experiment 2 were analyzed statistically using ANOVA, and the results are shown in Table 3.

Analysis of Coaptation Behavior

In Experiment 3, the number of monos and triominoes was recorded in addition to the position of the two monos and the triomino, as shown in Table 4. The number of monos and triominoes was also recorded in Experiment 4, as shown in Table 5. The data obtained in Experiment 4 were analyzed statistically using ANOVA, and the results are shown in Table 6.

In Experiment 5, the number of monos and triominoes was recorded in addition to the position of the two monos and the triomino, as shown in Table 7. The number of monos and triominoes was also recorded in Experiment 6, as shown in Table 8. The data obtained in Experiment 6 were analyzed statistically using ANOVA, and the results are shown in Table 9.
latory interval, from ejaculation to the next mount, was recorded. In Experiment 2, latency from the introduction of the female until the first ejaculation was recorded. This experiment only included males that ejaculated with the first intromission without preceding mounts.

Analysis of Fos-IR

Numbers of Fos-positive neurons were counted in MPN, MEApd, BNSTpm, PD, and SPFp, brain regions in which Fos-IR is induced following copulation (8). Areas analyzed are depicted in Fig. 1. Subdivisions within these brain regions that express Fos-IR following ejaculation, but not following intromissions (9), were analyzed separately and are presented in Fig. 1 as black areas. Therefore, in MEApd, Fos-IR cells were counted in a lateral and medial subdivision. Likewise, in BNSTpm, counts were made in a rostral and caudal subdivision. In the latter, cell counts were performed in a ventral and dorsal portion. Fos-IR neurons were counted unilaterally in two adjacent sections from each brain region in each rat by an observer blind to the treatment groups, using a Leitz light microscope attached to a Gateway personal computer running Neurolucida 2.1. For all subjects, Fos-IR neurons were quantified in a standard area of 0.25 mm² (MPN), 0.125 mm² (SPFp), 0.1 mm² (lateral MEApd, medial MEApd, caudal BNSTpm dorsal portion), or 0.04 mm² (rostral BNSTpm, caudal BNSTpm ventral portion, PD). Results from two cell counts in each animal were averaged and data are presented as mean (±SEM) Fos-IR cells in each group. Numbers of Fos-positive cells in the eight brain regions were compared using a two-way ANOVA and post hoc comparisons were made using the Scheffé test, both with a 0.05 level of significance. In Experiment 2, data for the control group included only counts from one animal in the lateral and medial MEApd and SPFp (shown in Table 2). Therefore, in these brain areas, Fos-IR following mating in males treated with the high dose of drug in Experiment 2 was compared to Fos-IR in the nonmating control animals treated with a low dose of
drug from Experiment 1. This was done only after it was determined that there was no overall effect of the different doses of 8-OH-DPAT in these three brain regions. Additionally, no differences were observed in control animals treated with high or low dose of the drug in the other brain regions. Mating behavior was analyzed based on medians using the Mann–Whitney U test with a 0.05 level of significance.

RESULTS

Behavioral Effects of 8-OH-DPAT

In agreement with previous studies, symptoms of the “5-HT syndrome” were observed in all 8-OH-DPAT-treated males, including lower lip retraction, flat body posture, forepaw treading, and abducted hind limbs (4,18,40). In addition, 8-OH-DPAT decreased exploratory behavior and other general activity compared to the saline-treated males (22). Moreover, males treated with 8-OH-DPAT exhibited no genital grooming following intromissions or ejaculations and displayed little chemosensory investigation of the females.

8-OH-DPAT facilitated ejaculation as shown in Table 1. Most importantly, the average total number of mounts and intromissions preceding the two ejaculations was significantly lower following 8-OH-DPAT (respectively 4 ± 1 vs. 14 ± 4 and 6 ± 2 vs. 27 ± 2; \( p = 0.0495, U = 0 \)). Also, average latency to the first ejaculation in males treated with the lower dose of 8-OH-DPAT in Experiment 1 was significantly shorter compared with that in saline-treated males (102 ± 31 vs. 777 ± 51 s; \( p = 0.0495, U = 0 \)). However, the average latency to the first intromission was longer following 8-OH-DPAT treatment (24 ± 5 vs. 4 ± 1 s and 27 ± 7 vs. 8 ± 2 s; \( p = 0.0495, U = 0 \)). In Experiment
2, in which males were treated with a higher dose of 8-OH-DPAT, ejaculation occurred with the first intromission, without previous mounts, at an average of 18.3 s after presentation of the female.

Distribution of Fos-IR: Experiment 1

Mating behavior that included two ejaculations induced a distinct distribution of Fos-IR in neuronal clusters in BNSTpm, PD, MEApd, and SPFp in both 8-OH-DPAT-treated males (DPAT-M) and saline-treated (SAL-M) males, despite the difference in sexual activity preceding the ejaculations (Fig. 2–4). In the BNSTpm, two Fos-IR clusters were present following mating, one being situated rostrally, close to the lateral ventricle (rostral BNSTpm; Fig. 2A–C), and the second located more caudally, lateral to the fornix, in the ventral portion of the caudal BNSTpm (caudoventral BNSTpm; Fig. 2D–F). In MEApd, a dense population of Fos-IR neurons was situated in the lateral subdivision of the MEApd (lateral MEApd; Fig. 4A–C). In addition, clusters of Fos-IR neurons were present in the medial part of the SPFp (Fig. 4D–F) and in the PD (Fig. 3). This distribution of Fos-IR was similar to that reported previously following mating including ejaculation (9) and was never observed in nonmating saline-treated (SAL-C) or 8-OH-DPAT-treated (DPAT-C) males.

In these brain regions, i.e., rostral BNSTpm, caudoventral BNSTpm, PD, lateral MEApd, and SPFp, mating behavior that included two ejaculations significantly increased the number of Fos-IR neurons in both SAL-M and DPAT-M males (Fig. 5 and 6; F(1, 8) = 60.4–114.0; p < 0.0001). Moreover, there were no differences between DPAT-M and SAL-M males in the numbers of Fos-IR neurons, except in PD, where numbers of Fos-IR neurons were significantly lower in DPAT-M males compared to SAL-M males (p = 0.0313; Fig. 6).

In addition, mating increased Fos-IR in MPN and medial MEApd in both SAL-M and DPAT-M males (Fig. 7; F(1, 8) = 106.8–124.7; p < 0.0001). However, the numbers of Fos-IR
neurons were significantly lower in DPAT-M males compared to SAL-M males ($p = 0.0045$ and $p = 0.0021$, respectively; Fig. 7). In the dorsal portion of the caudal BNSTpm (caudodorsal BNSTpm), Fos-IR was only increased in SAL-M males ($p = 0.0228$; Fig. 7).

Administration of 8-OH-DPAT in nonmating control males (DPAT-C) did not affect the number of Fos-IR cells in MPN, rostral BNSTpm, caudodorsal BNSTpm, PD, lateral MEApd, or medial MEApd (Fig. 5–7). However, the numbers of Fos-IR neurons in the caudoventral BNSTpm and SPFp of DPAT-C males were significantly lower than that of SAL-C males ($p = 0.0286$ and $p = 0.034$, respectively; Fig. 5–7).

**Distribution of Fos-IR: Experiment 2**

As noted earlier, following a high dose of 8-OH-DPAT, males ejaculated during the first intromission without any preceding mounts. Despite the limited sexual activity preceding the ejaculation, clusters of Fos-positive neurons similar to those observed after the lower dose of 8-OH-DPAT in Experiment 1 were evident in the rostral and caudoventral BNSTpm, lateral MEApd, and SPFp (Fig. 8). However, Fos-IR clusters were not seen in the PD of these animals. Accordingly, one ejaculation induced by the high dose of 8-OH-DPAT increased the numbers of Fos-IR neurons in the rostral and caudoventral BNSTpm ($F(1, 8) = 280.349–137.146; p < 0.0001$), lateral MEApd ($F(1, 6) = 27.467; p = 0.0019$), and SPFp ($F(1, 6) = 34.606; p = 0.0011$), but not in PD, as shown in Table 2. Moreover, in the lateral MEApd and SPFp, the numbers of Fos-IR neurons activated by one ejaculation following the high dose of 8-OH-DPAT were not different from Fos-IR following two ejaculations in males treated with a lower dose of 8-OH-DPAT (Table 2). However, in the rostral and caudoventral BNSTpm, the numbers of Fos-IR cells were significantly lower following one ejaculation in males treated with the high dose...
than following two ejaculations in DPAT-M males treated with the low dose of 8-OH-DPAT (p = 0.0017 and p = 0.0099, respectively). In addition, Fos-positive neurons in the lateral MEApd, rostral and caudoventral BNSTpm, and SPFp in males that displayed one ejaculation appeared more lightly stained than those in males in Experiment 1 that displayed two ejaculations. However, the intensity of Fos-immunostaining in other brain areas that showed basal levels of Fos-IR, including the endopiriform nucleus, and the paraventricular nucleus of the thalamus, appeared to be comparable in all animals.

One ejaculation without preceding mounts also increased Fos-IR in MPN (F(1, 8) = 14.657; p = 0.005), which was not significantly different from Fos-IR following two ejaculations in DPAT-M males treated with the lower dose of 8-OH-DPAT. Administration of the high dose of 8-OH-DPAT in nonmating control animals did not affect Fos-IR in any of the studied brain areas (Table 2).

DISCUSSION

The present study describes neural activation in male rats specifically following ejaculation with the use of the 5-HT1A agonist 8-OH-DPAT. Administration of 8-OH-DPAT facilitated ejaculatory behavior, resulting in a low number of mounts and intromissions preceding ejaculation. Nonetheless, mating in 8-OH-DPAT-treated animals resulted in a similar pattern of Fos-IR neurons as in mated, saline-treated males. Specifically, in both groups of animals Fos-positive neurons were present in discrete clusters in the BNSTpm, MEApd, PD, and SPFp. Administration of 8-OH-DPAT by itself in nonmating control males did not increase neural activity in these brain regions. Therefore, expression of Fos following mating in males that were treated with this drug is likely to reflect neural activation specific to ejaculation. Together with our previous finding that intromissions alone do not induce the presence of the characteristic clusters of Fos-IR neurons (9), the present results suggest the existence of a specific ejaculation-related subcircuit within a larger circuit involved in male sexual behavior.

In recent years our laboratory and others have used Fos-IR to map neural circuits underlying sexual behavior in male rodents (3,8,9,15,20,25,27,42). Mating increases Fos-IR in brain regions that have been implicated in control of sexual behavior and where, in most instances, lesions have been shown to disrupt the behavior (30). However, an advantage of the use of Fos as a marker is a cellular resolution that lesions cannot provide. As a result of this greater resolution, activated neurons were observed following mating in subregions within the brain areas involved in sexual behavior in male rats (9), hamsters (15,25), and gerbils (20), including MPN, BNSTpm, MEApd, PD, and SPFp. Moreover, discrete clusters of Fos-IR were only observed in specific portions within these subregions following ejaculations and not following intromissions (9,20). In the rat, these clusters of Fos-IR neurons were observed in the lateral zone of the MEApd, the rostral and caudoventral portions of BNSTpm, PD, and the medial portion of SPFp. Except for PD and the lateral zone of the MEApd these subregions have not been distinguished on Nissl-stained sections or previously defined as anatomically separate regions. Display of intromissions without ejaculation is not sufficient to induce these Fos-IR clusters, but this finding itself does not establish if this particular pattern of neural activation is specific to ejaculation per se, since it may reflect accumulated sexual activity preceding ejaculation. Therefore, the present study used an opposite approach, studying Fos-IR following pharmacologically induced ejaculation. The results demonstrate that the Fos-IR clusters in BNSTpm, PD, MEApd, and SPFp are specific to ejaculation, thereby further supporting the existence of an ejaculation-specific subcircuit.

Interestingly, although ejaculation is necessary to induce the Fos-IR clusters (9), the sufficiency of ejaculation alone to activate Fos-positive cells does not appear to be equal for all these subregions. Following a single ejaculation without previous sexual activity, Fos-IR was present in rostral and caudoventral BNSTpm, lateral MEApd, and SPFp, but no Fos expression was evident in
The other brain regions containing ejaculation-related neural activity receive ascending genital or visceral sensory inputs directly or contiguously, and containing ejaculation-related Fos-IR separately do not completely abolish display of ejaculatory behavior, indicating that the individual clusters are not primarily required for triggering ejaculation (30). Although, in contrast, a recent study of gerbils indicated that lesions of MEApd, but not of PD, disrupted copulation. This suggests that MEApd in male gerbils could be involved in control of ejaculation (21).

The precise function of the ejaculation-related neuronal clusters remains to be determined. A common question in studies using Fos as a marker of activation is whether the observed Fos expression is due to sensory stimulation, behavioral output, or both. With respect to studies of mating behavior, accumulated evidence suggests that mating-related Fos is induced by sensory and hormonal inputs (3,43), rather than being triggered by behavioral display. Accordingly, neural activation in the ejaculation-related subcircuit appears consistent with genital sensory signals. In female rats, clusters of Fos-positive neurons that are similar to the ejaculation-specific clusters in males are present following vaginocervical stimulation (9,13,35,39). Furthermore, in females, as in males, the pelvic nerve relays visceral sensory input from the internal reproductive organs (5,11). Transection of the pelvic nerve reduced ejaculation-related neural activation in the brain regions containing the clusters in both male and female rats (36,42). Thus, in a similar manner, we hypothesize that ejaculation-related neural activation in male rats is related to visceral sensory stimulation relayed via the pelvic nerve. Moreover, in the male rat and hamster, lesions in each of the subnuclei containing ejaculation-related Fos-IR separately do not completely abolish display of ejaculatory behavior, indicating that the individual clusters are not primarily required for triggering ejaculation (30).

The neuroanatomical connections of the regions that contain ejaculation-related clusters is consistent with the view that this activation is due to sensory stimulation. For example, the SPFp receives ascending genital or visceral sensory inputs directly or indirectly from the lumbo-sacral spinal cord (23,28,32). Although the other brain regions containing ejaculation-related neural activity are not known to receive direct projections relaying genital sensory cues, they form a heavily interconnected network through which these signals may be relayed. The SPFp projects to MEApd (25,41,44), which in turn is heavily interconnected with BNSTpm (6,24,26). Furthermore, all the brain regions containing ejaculation-related Fos-IR clusters have reciprocal connections with MPN (6,24,26,38), a brain region involved in the regulation of copulation (14,30).

Interestingly, studies in hamsters have indicated a role for ejaculation-related neural activity in sexual satiety (33). Activated neurons in the lateral MEApd are only observed following multiple ejaculations or when hamsters are reaching sexual satiety, following only a few ejaculations due to mating on consecutive days. Moreover, a recent study demonstrated that lesions of MEApd progressively increased the number of ejaculations necessary for reaching satiety on the third consecutive day of mating (34). Although rats differ from hamsters in the sense that neural activation in MEApd and in the other subregions is already present following a single ejaculation, it remains possible that the ejaculation-related neuronal clusters are involved in mediating sexual satiety or the postejaculatory interval. In conclusion, the present results provide additional support for the existence of an ejaculation-specific subcircuit within the larger neural circuits underlying male sexual behavior. Neural activation in the involved brain regions appears to reflect visceral or genital sensory inputs. However, the precise function(s) of this subcircuit remains to be determined in further investigation.

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