Tail Pinch Induces Sexual Behavior in Olfactory Bulbectomized Male Rats

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Received 24 August 1979

WANG, L. AND E. M. HULL. Tail pinch induces sexual behavior in olfactory bulbectomized male rats. PHYSIOL.
BEHAV. 24(2) 211-215, 1980.—Adult male Long-Evans rats were subjected to bilateral olfactory bulbectomy, sham
surgery or no treatment. Of 34 bulbectomized rats, 24 failed to ejaculate on either of 2 tests with a primed ovariec-
tomized female. All control animals exhibited normal sexual behavior, and 10 bulbectomized animals ejaculated at least once during
the 2 tests. Later histological examination revealed a relationship between size of lesion and extent of behavioral deficits.
After a third test, 16 nonejaculatory animals were subjected to a tail pinch (TP) procedure, immediately followed by a
fourth test. The remaining 8 nonejaculatory animals were tested similarly, but without tail pinch. Ten of the 16 tail pinch
animals showed complete sexual behavior on the first test, while 2 additional animals began to copulate after a second TP
procedure 4 days later. Only 1 of the 8 animals not receiving TP ejaculated on these tests. Thus, TP applied shortly before
sexual behavior tests can induce copulation in some males whose behavior had been disrupted by olfactory bulbectomy.

SEVERAL studies have found that bilateral olfactory bulbectomy in rats leads to alterations in such behaviors as copula-
tion [10, 16, 19], mouse killing [1, 17, 32], intermale ag-
gression [5], and maternal behavior [14,15]. Several lines of
evidence indicate that the observed changes following bulb-
ectomy are not solely attributable to the loss of olfactory
cues. Bulbectomized animals have been reported to be im-
paired in visual discrimination problems [24] and in passive
and active avoidance tasks [20,29], to exhibit altered sponta-
nous behavior patterns [30,31], and to be more irritable and emotional [1, 5, 12, 13]. The contribution of the olfactory
sense to these behaviors is not immediately obvious. In ad-
dition, the effects of olfactory bulbectomy and peripheral
olfactory manipulations (such as intranasal zinc sulfate
treatment or surgical ablation of the receptors) are not iden-
tical, in that some behavioral changes and learning deficits
observed in bulbectomized animals were absent in the an-
imals with peripheral lesions.

Bilateral olfactory bulbectomy in sexually experienced rats leads to prolonged response latencies and loss of ability
to ejaculate [10,16], and in decreased intromissions [10]. In
these studies bulbectomized animals were reported to ex-
hibit a marked variation in copulatory pattern from test to
test, and to show little or no interest in the receptive females.
Cain and Paxinos [10] proposed that copulatory deficits fol-
lowing bilateral olfactory bulbectomy may be due to changes
in the central arousal mechanism. Preoperative sexual expe-
rience seems to be an important factor in whether mating
behavior is displayed postoperatively [6,19]. In these studies
more severe deficits were produced in the inexperienced
than in the experienced animals.

A tail pinch procedure has been shown to elicit a variety
of behaviors, such as eating [2,26], copulation [9], and ma-
ternal behavior [28,35] in the presence of appropriate goal
objects. Similarly, shock to the tail was reported to elicit
copulation or aggression, depending on the test situation [8].
The mechanism underlying these effects has been hypo-
thesized to be an arousal induced lowering of dopaminergically
mediated sensorimotor thresholds [2].

The present study investigated whether tail pinch-induced
arousal could overcome any copulatory deficits resulting
from olfactory bulbectomy. The first experiment analyzed
the copulatory pattern in sexually inexperienced rats follow-
ing bulbectomy and correlated behavioral deficits with ex-
tent of lesion to the bulbs. The second experiment tested the
effects of tail pinch in reversing the copulatory deficits in
bulbectomized male rats.

EXPERIMENT 1

METHOD

Animals

Forty-one male Long-Evans rats were purchased from

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Charles River Laboratories when 11 weeks old. Animals were housed in individual metal cages and maintained on a 14:10 light:dark cycle. Food and water were available ad lib. Thirty-four were subjected to bilateral olfactory bulbectomy (BOB); 4, to sham operations (SO); and 3 served as intact controls (IC).

Surgery

At the time of surgery animals were 12 weeks old and weighed 300-380 g. Surgery was performed under Diabutal anesthesia (50 mg/kg), preceded by atropine sulfate (1 mg/kg). After placement of the animal in a stereotaxic apparatus, an incision was made through the scalp, and the skull overlying the olfactory bulbs was drilled down to the dura. Dura was penetrated with a sharp needle. Under the guidance of a magnifying lens, the olfactory bulbs were aspirated and the upper aspect of the cribriform plate was scraped. Gel Foam was used to effect hemostasis and sulfathiazole was sprinkled on the wound to minimize infection. After the wound was sutured, any blood present in the external nares and oral cavity was aspirated. Animals were allowed to recover for two weeks before the behavioral tests. Four sham operated animals received similar treatment except that no olfactory structure was removed.

Sexual Behavior Tests

Six ovariectomized females were brought into estrus by 2 subcutaneous injections of estradiol benzoate (10 µg/kg) followed by an injection of progesterone (0.5 mg) 8 hr before testing. All testing was done in the dark phase of the light-dark cycle. One hour after light offset an animal was brought into the observation cage (a 10-gal aquarium, 51x27x30 cm) and allowed to adapt for 10 min. A receptive female was then introduced into the cage. Both copulatory and noncopulatory behaviors were recorded during the 40 min observation period. Noncopulatory behavior included licking the female on the neck or shoulder, sniffing the ano-genital area, chasing and fighting. Copulatory behavior was scored according to the following 6 categories:

Mount latency. Time from the introduction of the female to the first mount.

Intromission latency. Time from the introduction of the female to the first intromission.

Ejaculation latency. Time from the first intromission to the first ejaculation.

Mount/ejaculation ratio (M/E). Average number of mounts to achieve one ejaculation.

Intromission/ejaculation ratio (I/E). Average number of intromissions to achieve one ejaculation.

Ejaculations. Number of ejaculations achieved during the 40 min observation.

A score of 40 was assigned to either Mount Latency or Intromission Latency if the subject failed to show either of those behaviors. When ejaculations were not observed, the M/E, I/E, and Ejaculation scores were not determined. A second test was administered after a 4 day interval to those animals which failed to ejaculate during the first test.

Histology

After all behavioral tests had been completed, animals were perfused intracardially with saline and Formalin, and the brains were removed and exposed to Formalin. Brains were examined visually and rated according to the extent of damage. They were then embedded in paraffin, cut into 40 µ sections, and stained with cresyl violet. Surgical damage was then rated separately for each side on a 4 point scale and the two ratings were summed. Rating was done without knowledge of behavioral results. Ratings of both whole brains and sections utilized the following scale: 1= incomplete removal of main olfactory bulb (MOB); 2=complete removal of MOB, but anterior olfactory nucleus (AON) largely intact; 3=significant damage to AON; 4=AON removed, but olfactory tubercle intact.

RESULTS

Twenty-four of the 34 BOB rats exhibited either incomplete or no copulatory behavior in postoperative tests. Of these impaired animals, 3 showed mounting without intromission or ejaculation; 2 mounted and intromitted but failed to ejaculate; the other 19 animals did not mount, intromit, or ejaculate during either test. The remaining 10 BOB animals ejaculated at least once during the two tests. While all animals in the IC and SO control groups were able to display the full spectrum of copulatory behavior in all the tests, BOB animals showed severe impairment on most of the tests. Mounting was observed in 35% of tests of BOB animals, intromission, in 28%, and ejaculation, in 25% of the tests. The differences between BOB and the combined control groups (IC and SO) were highly significant (Mount: x²(1)=19.54, p<0.005, Intromission: x²(1)=25.10, p<0.005, Ejaculation: x²(1)=27.79, p<0.005).

The mean number of mounts, intromissions, and ejaculations are summarized in Table 1. BOB animals achieved significantly fewer mounts, F(2,38)=10.95, p<0.01, intromissions F(2,38)=7.46, p<0.05, and ejaculations, F(2,38)=15.82, p<0.01, per test compared with either IC or SO animals, using Newman-Keuls comparisons. The 2 control groups did not differ from each other.

The data of BOB animals which showed at least one ejaculation (BOB/ND: no deficit) were analyzed separately from those which failed to ejaculate (BOB/D: deficit). The results of this comparison are summarized in Table 2. Copulatory scores of BOB/D animals were significantly different from the other 3 groups on all measures. The only differences among the other 3 groups were decreased number of ejaculations by the BOB/ND group compared to the 2 control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mounts</th>
<th>Intromissions</th>
<th>Ejaculations</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOB</td>
<td>34</td>
<td>5.47 ± 1.26</td>
<td>6.51 ± 1.58</td>
<td>0.57 ± 0.13</td>
</tr>
<tr>
<td>SO</td>
<td>4</td>
<td>25.35 ± 5.71</td>
<td>27.52 ± 3.64</td>
<td>2.93 ± 0.26</td>
</tr>
<tr>
<td>IC</td>
<td>3</td>
<td>20.83 ± 4.01†</td>
<td>26.00 ± 3.12§</td>
<td>3.00 ± 0.36#</td>
</tr>
</tbody>
</table>

*BOB vs. SO, q(3,38)=5.36, p<0.01.
†BOB vs. IC, q(2,38)=3.87, p<0.01.
§BOB vs. IC, q(2,38)=3.71, p<0.05.
#:BOB vs. SO, q(2,38)=5.36, p<0.01.
#BOB vs. IC, q(3,38)=5.52, p<0.01.
TABLE 2
MEANS ± STANDARD ERRORS OF COPULATORY BEHAVIOR SCORES

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mount lat. (min) ± SE</th>
<th>Intromission lat. (min) ± SE</th>
<th>Ejaculation lat. (min) ± SE</th>
<th>M/E ± SE</th>
<th>I/E ± SE</th>
<th>Ejaculations ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOB/D</td>
<td>24</td>
<td>35.47 ± 1.76</td>
<td>39.22 ± 0.61</td>
<td>40.00 ± 0.00</td>
<td>—</td>
<td>—</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>BOB/ND</td>
<td>10</td>
<td>5.54 ± 2.14</td>
<td>8.71 ± 2.57</td>
<td>17.46 ± 2.49</td>
<td>8.39 ± 1.69</td>
<td>9.55 ± 1.45</td>
<td>1.95 ± 0.23</td>
</tr>
<tr>
<td>SO</td>
<td>4</td>
<td>3.82 ± 2.41</td>
<td>6.28 ± 2.69</td>
<td>12.50 ± 2.16</td>
<td>7.97 ± 1.33</td>
<td>8.37 ± 3.87</td>
<td>2.93 ± 0.26</td>
</tr>
<tr>
<td>IC</td>
<td>3</td>
<td>3.90 ± 2.09</td>
<td>6.71 ± 2.81</td>
<td>11.65 ± 3.29</td>
<td>7.82 ± 2.02</td>
<td>9.18 ± 1.20</td>
<td>3.00 ± 0.36</td>
</tr>
</tbody>
</table>

All analyses of variance were significant (p<0.01). Paired comparisons indicated that BOB/D animals differed from all other groups on all measures (p<0.01). BOB/ND animals also showed fewer ejaculations than the SO and IC groups (p<0.05 for both).

TABLE 3
EFFECT OF TP ON COPULATORY SCORES OF BOB/D ANIMALS

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mount lat. ± SE</th>
<th>Introm. lat. ± SE</th>
<th>Ejac. lat. ± SE</th>
<th>M/E ± SE</th>
<th>I/E ± SE</th>
<th>Ejaculations ± SE</th>
<th>% tests with ejac.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP*</td>
<td>12</td>
<td>2.42 ± 1.17</td>
<td>6.66 ± 1.86</td>
<td>8.45 ± 1.46</td>
<td>3.64 ± 0.33</td>
<td>9.24 ± 1.76</td>
<td>2.67 ± 0.19</td>
<td>91.7</td>
</tr>
<tr>
<td>TP†</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.00 ± 0.00</td>
<td>00.0</td>
</tr>
<tr>
<td>TP‡</td>
<td>16</td>
<td>9.34 ± 3.90</td>
<td>15.03 ± 3.97</td>
<td>16.34 ± 3.69</td>
<td>—</td>
<td>—</td>
<td>2.00 ± 0.33</td>
<td>68.8</td>
</tr>
<tr>
<td>NTP§</td>
<td>1</td>
<td>19.67</td>
<td>20.33</td>
<td>9.33</td>
<td>0.50</td>
<td>6.5</td>
<td>2.00</td>
<td>100.0</td>
</tr>
<tr>
<td>NTP#</td>
<td>7</td>
<td>34.56 ± 5.44</td>
<td>38.67 ± 1.33</td>
<td>40.00 ± 0.00</td>
<td>—</td>
<td>—</td>
<td>0.00 ± 0.00</td>
<td>00.0</td>
</tr>
<tr>
<td>NTP#</td>
<td>8</td>
<td>32.70 ± 5.42</td>
<td>36.38 ± 2.74</td>
<td>36.17 ± 4.10</td>
<td>—</td>
<td>—</td>
<td>0.13 ± 0.13</td>
<td>6.2</td>
</tr>
</tbody>
</table>

*Animals induced by TP to copulate.
†Animals not induced by TP to copulate.
‡Total of all TP animals.
§Animal which ejaculated without TP.
¶Animals which did not receive TP and did not ejaculate.
#Total of all animals which did not receive TP.

In the IC and SO control animals the first mount was usually preceded by brief sniffing of the female's ano-genital area, while the postintromission and postejaculatory behavior was characterized by the male's genital licking and grooming of himself. In contrast, behaviors evident during the encounter between the female and the BOB/D males were dominated by sniffing of the female's shoulder or back, pressing on the female's back with the forepaws, biting the female's neck and flank, and subsequent chasing of the female. Other inappropriate behavior included bumping into the female and mounting from her side. Each female, on the other hand, was found to display soliciting behavior toward each male with which she was tested, exhibiting lordosis in response to the male's pressing and turning her back toward the male when he approached. Successful copulation was characterized by a brief encounter between male and female, while unsuccessful mating was dominated by interfering behavior and substantially longer physical interaction between the two.

EXPERIMENT 2

METHOD

The BOB animals which had showed a sexual deficit in Experiment 1 were subjected to a tail pinch (TP) procedure to determine whether it would facilitate sexual performance. To control for the possibility that increased copulation seen during TP tests was due to behavioral recovery or variation in these deficits, 16 of the BOB/D animals were randomly assigned to the TP group while the remaining 8 animals were repeatedly tested at 4-day intervals without TP.

The tail pinch procedure consisted of 6 applications of an alligator clip (Pee Wee No. 45, Mueller Elect. Co., Cleveland, O) approximately 2 cm from the tip of the tail. Each application was approximately 60 sec, with a 2-3 min interval between applications. The tail fit into an elliptical hole near the end of the clip which was 3 x 5 mm when the clip was closed. The clip was attached to a 12 cm length of elastic by means of which the end of the tail could be kept out of the animal's reach. During pilot trials with other animals food biting and/or drinking and licking of the water dish could be obtained within 5-6 applications. Nonconsummatory behaviors which were also elicited included biting the clip, teeth chattering, urination, defecation, and vocalization, as well as grooming, exploring, sniffing, licking the floor, backward walking, rearing, and food retrieving.

All BOB/D animals were given a third copulatory behavior test 4 days following the second test of Experiment 1. No animal ejaculated on this test. Sixteen of the 24 were then subjected to the tail pinch procedure. The female was removed from the observation cage and the male was given...
approximately 6 applications of the clip, as described above. The female was reintroduced and a new 40-min test was begun. The remaining 8 BOB/D animals were subjected to a similar procedure, except without application of the clip. If the animal failed to ejaculate during this test, the entire tail pinch procedure was repeated 4 days later, including pre- and post-TP copulatory behavior tests.

RESULTS

Of the 16 BOB/D animals tested with TP, 10 were induced to mount, intromit, and ejaculate on the first test following TP. Two additional animals began to show the full range of copulatory behavior during the second TP test 4 days later (see Table 3). TP was effective in inducing ejaculation in 91.7% of the tests of the animals which did ejaculate, while the combined percentage of ejaculatory trials of all 16 animals receiving TP was 68.8. The mounts, intromission and ejaculation latencies and ratios of the 12 animals which were induced to copulate were compared with the BOB/ND, SO and IC groups of Experiment 1. The animals showing TP-induced copulation had shorter ejaculation latencies than the BOB/ND group, F(3,25)=3.02, p<0.05; paired comparison, F(1,25)=8.90, p<0.01, and more ejaculations than that group, F(3,25)=3.08, p<0.05; paired comparison F(1,25)=5.55, p<0.05. They also exhibited fewer mounts per ejaculation than the BOB/ND group, F(3,25)=3.05; p<0.05; paired comparison F(1,25)=11.02, p<0.01, and than all other groups combined, F(1,25)=8.80, p<0.01. One BOB/D animal which did not receive TP ejaculated on the first of these tests. Another BOB/D animal tested without TP showed some mounts and intromissions, but these behaviors were usually interspersed with inappropriate behaviors and did not lead to ejaculation.

Extent of surgical damage to BOB/D was significantly greater than that to BOB/ND according to analysis of whole brain ratings, Kruskal-Wallis H (1)=4.11, p<0.05. However, ratings of histological sections revealed only a trend in the same direction, H (1)=3.09, 0.05<p<0.1.

GENERAL DISCUSSION

This study confirms earlier findings that bilateral olfactory lesions lead to severe impairment in the mating behavior of sexually inexperienced male rats [6, 7, 16, 19]. The presence of behavioral deficits bore some relationship to the extent of damage to the bulbs. Apparently a small fraction of the normal complement of functioning olfactory structure can be sufficient for normal copulatory behavior to occur. This point is supported by observations that unilaterally bulbectomized animals can function as well as normal animals in most olfactory-mediated behavioral tasks (e.g. [25]). The presence of some residual functioning structure might also explain the partial deficits reported in many studies [7, 10, 16, 19, 33, 34].

Different species seem to differ in the degree to which copulatory behavior is dependent upon olfaction. Hamsters, for example, have been found to be more dependent upon olfaction than rats and mice. In the former, copulation is totally abolished following bilateral olfactory bulbectomy, regardless of the animal's preoperative experience [22]. In rats, however, preoperative experience tended to protect sexual behavior patterns from disruption by the surgery [6, 19].

Our bulbectomized rats with sexual deficits exhibited two quite dissimilar behavior patterns. Some showed no interest at all in the receptive female, while others showed strong interest in sexually unrelated stimuli and exhibited many interfering behaviors. It is not clear whether differential damage to olfactory structures can account for these behavioral differences.

It has been demonstrated that copulatory impairment following bulbectomy is not due to androgen deficit [19, 22, 25]. Different mechanisms have been proposed to account for the impairment, among which is disruption of a central arousal mechanism [10]. Our results are consistent with this hypothesis in that stimulation of the tail, which is generally considered to be an arousing stimulus, was sufficient to elicit the full spectrum of copulatory behavior in previously sexually impaired bulbectomized animals. The TP procedure seems to have brought the animal into a more efficient state so that ejaculation latencies were shorter. Fewer mounts preceded ejaculation, and more ejaculations were obtained compared with BOB animals copulating without TP. After this study was completed we became aware of an experiment demonstrating induction by intermittent flank shock of sexual behavior in male rats bulbectomized neonatally or in adulthood [27]. These animals did not copulate prior to the shock procedure nor in subsequent tests without it. These results are also congruent with the arousal hypothesis.

TP has been reported to elicit stimulus bound behaviors such as eating [2,26], maternal behavior [28,35], and sexual behavior [9]. It was also found to facilitate learning of a T-maze [18]. The behavioral effect of TP seems to depend on an intact nigrostriatal dopamine system [3]. It should be noted that Antelman et al. [2,4] and Marques et al. [21] reported that TP induced food pellet eating, biting, and gnawing but failed to induce rats to drink tap water from burettes. In our pilot tests of the TP procedure 4 intact, nondeprived male rats were subjected to TP in the presence of food pellets and a shallow cup of water. (They normally drank from standard laboratory water bottles.) Some of the animals not only began to drink water during TP, but did so on an earlier trial than they chewed food pellets. Drinking was sometimes accompanied by extensive licking of the edge of the cup. In some trials alternate drinking and food biting was observed for up to 45 sec. Since consumatory behaviors were not our major concern, we did not measure amount of food or water ingested.

It is important to note that the sexual behavior observed in Experiment 2 occurred after removal of the clip from the tail. Thus, the relatively long lasting stimulation distinguishes the TP effect from that of brain stimulation [23], which elicits behavior patterns only during the period of stimulation. Some of the animals which had been induced to copulate were later given additional tests without TP, and were observed to copulate on these tests. Caggiula et al. [9] and Szechtman et al. [35] also reported stimulatory effects of TP on copulatory and on maternal behaviors, respectively, which outlasted the period of application.

Our finding that TP can induce copulatory behavior in bulbectomized animals which had previously exhibited either no interest in the female or interest in sexually irrelevant cues, suggests that TP may exert its effect by stimulating an arousal mechanism and/or directing the animal's attention to sexually-related cues.
REFERENCES


