Published in final edited form as:

Horm Behav. 2009 January ; 55(1): 93–97. doi:10.1016/j.yhbeh.2008.08.012.

Sexual reward in male rats: Effects of sexual experience on conditioned place preferences associated with ejaculation and intromissions

Christine M. Tenk^{1,*}, Hilary Wilson^{3,*}, Qi Zhang¹, Kyle K. Pitchers^{1,2}, and Lique M. Coolen^{1,2}

¹ Department of Physiology & Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada, N6A 5C1

² Department of Anatomy & Cell Biology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada, N6A 5C1

³ Neuroscience Graduate Program, University of Cincinnati, Cincinnati, Ohio

Abstract

Various behavioral models and studies have provided evidence suggesting that male rat sexual behavior has rewarding and reinforcing properties. However, there is little information regarding the rewarding values of the different components of sexual behavior. Therefore, this study used a conditioned place preference (CPP) paradigm to address whether ejaculation and intromissions differ in their rewarding incentive values. We also addressed whether the differential rewarding values were dependent on prior sexual experience. Sexually naïve and experienced males received one pairing of either intromissions or ejaculation with one of the chambers in the CPP box. The amount of time spent in each chamber of the CPP apparatus after conditioning was then measured. Both sexually naïve and sexually experienced males formed a CPP for ejaculation, while, only sexually naïve, and not sexually experienced, males formed a CPP for intromissions. Moreover, in sexually naïve males, multiple pairings of ejaculation with the designated chamber resulted in a CPP relative to the control chamber paired with display of intromissions. These data support the hypothesis that there is a hierarchy of rewarding sexual behavior, with ejaculation being the most rewarding component, and that the rewarding incentive value of other components of sexual behavior is dependent upon prior sexual experience.

Keywords

reward; conditioned place preference; copulation; sexual behavior; associative learning

Corresponding Author: Lique M. Coolen, Phone: (519) 661 – 2111 ext. 80285, Fax: (519) 661-3827, Email: lique.coolen@schulich.uwo.ca.

Mailing address: Department of Anatomy & Cell Biology, Schulich School of Medicine & Dentistry, University of Western Ontario, Medical Sciences Building, Room 460, London, Ontario, Canada, N6A 5C1

*These authors made equal contributions.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

In male rodents, sexual behavior is a rewarding and reinforcing behavior, composed of various elements, including anogenital investigation, mounts, intromissions, and ejaculation. Ejaculation appears to be the most reinforcing component of sexual behavior (Coolen et al., 2004; for review see Pfaus and Phillips, 1991). For example, in contrast to males allowed to only intromit or mount, but not ejaculate, males allowed to copulate to ejaculation developed faster running speeds in T-mazes (Kagan, 1955), straight-arm runway (Lopez et al., 1955), or hurdle climbing (Sheffield et al., 1951). In addition, ejaculation is essential for the formation of conditioned copulatory preferences. That is, the association of a novel odor with a receptive female leads to a preference for, as well as facilitated sexual behavior toward, a similarly scented female in males allowed to copulate to ejaculation, but not in males allowed to display intromissions without ejaculation (Kippin and Pfaus, 2001).

The rewarding aspects of copulation have been demonstrated using a conditioned place preference (CPP) paradigm (Pfaus and Phillips, 1991). The CPP paradigm measures approach responses to environmental stimuli that previously have been paired with reinforcing events and can be used to evaluate the incentive value of these rewarding events and the reward-related stimuli (Carr et al., 1989). The apparatus used to demonstrate CPP typically consists of distinctive compartments that are paired differentially with unconditional stimuli: one side is paired with copulation to ejaculation, while the other side is paired with nothing or a control manipulation. Indeed, male rats that are allowed to copulate to ejaculation form a preference for the compartment paired with this behavior (Agmo and Berenfeld, 1990; Martinez and Paredes, 2001). However, it is not known whether the development of CPP is dependent on display of ejaculation, or if display of intromissions is sufficient. We hypothesize that ejaculation is more rewarding compared to other elements of sexual behavior given the previous studies showing its greater incentive properties.

Thus, the current set of experiments examined whether ejaculation is more rewarding than the display of multiple intromissions using the CPP paradigm. Moreover, the influence of sexual experience on the rewarding value of intromissions or ejaculation was investigated.

Materials and methods

Animals

Adult male Sprague Dawley rats (250–350g) were obtained from Harlan laboratories (Indianapolis, IN, USA) or Charles River (Senneville, QC, Canada) and singly housed on a 12-hour reversed light/dark cycle (lights off 10 a.m.). Food and water were available at all times except during behavioral testing. Stimulus females were ovariectomized and implanted with subcutaneous 5% 17- β -estradiol benzoate Silastic capsules (1.98 mm inner diameter, 0.5 cm length, Dow Corning Corporation, MI, USA). Sexual receptivity was induced by subcutaneous injections of progesterone (500 μ g in 0.1 ml of sesame oil) approximately four hours prior to mating sessions. All procedures were approved by the Animal Care and Use Committee of the University of Cincinnati, University of Western Ontario Animal Care Committee, and conformed to NIH and CCAC guidelines involving vertebrate animals in research.

Conditioned place preference apparatus

The conditioned place preference (CPP) apparatus (Med Associates, Vermont, USA) consisted of two test chambers (28 $\times$ 22 $\times$ 21 cm) separated by a central compartment (13 $\times$ 22 $\times$ 21 cm). Chambers were differentiated by both visual and tactile cues. One test chamber had white walls and metal grid flooring, while the other had black walls and parallel bar flooring. The central

compartment consisted of grey walls and a smooth grey floor. Doors on both sides of the central compartment separated the chambers, and could be raised to allow the animals free movement throughout the apparatus, or lowered to confine them to a particular area.

Behavioral conditioning and testing

All testing took place in the dark phase (three to six hours after lights off). Initial baseline preferences were determined on day one of the experiment by a pretest in which each animal was given free access to all chambers of the CPP apparatus for 15 minutes. Subjects were videotaped, and time spent in each chamber was analyzed using the Microsoft Excel Custom Macro Program. An animal was considered present in a chamber if the entire trunk of the animal's body, including hips, was located in that chamber. The chamber in which the animal spent less time (the initially non-preferred side) was designated the sex-paired side, and the other side (the initially preferred side) was designated the control side. Conditioning took place on days two and three. During the sex conditioning day, animals mated with a receptive female in their home cage, then were immediately transferred to the sex-paired chamber in the CPP apparatus for 30 minutes. During the control conditioning day, animals were taken directly from their home cage and placed in the control chamber of the CPP apparatus for 30 minutes. Half the animals in each experiment were given the sex pairing on day two and placed in the control chamber on day three. The remaining animals were given the control pairing on day two and the sex pairing on day three. Following this single conditioning trial (consisting of the two conditioning days), the animals' preference for the chambers was reassessed using a posttest on the final, fourth, day, that was procedurally identical to the pretest.

Experimental design

Four experiments were performed. The conditioning design and group numbers for each experiment are depicted in Table 1. In the first experiment, sexually naïve males received either ejaculation (n=10) or intromissions (six-eight intromissions without ejaculation; n=11) paired with the initially non-preferred chamber while no sex behavior was paired with the initially preferred chamber. Display of ejaculation was determined based on the characteristic motor behavior the animal displays upon ejaculation, as well as the presence of vaginal plug in the female partner. In addition, a control group was added, consisting of males (n=10) that were exposed to the chambers of the CPP apparatus following handling and control manipulations, but without mating, and thus served as un-stimulated controls. In the second experiment, sexually experienced males were used. These males were mated to one ejaculation in five mating sessions prior to CPP conditioning. Only males that displayed ejaculation in three of these five sessions were included in this experiment. As in experiment 1, these sexually experienced males received conditioning with either ejaculation (n=10) or intromissions (six-eight intromissions without ejaculation; n=10) paired with the initially non-preferred chamber while no sex behavior was paired with the other chamber. In the third experiment, sexually naïve males (n=8) displayed ejaculation paired with the initially non-preferred chamber and intromissions (six-eight intromissions without ejaculation) paired with the other (control) chamber. Finally, in the fourth experiment, males that were sexually naïve prior to this experiment (n=10) displayed ejaculation or intromissions (six-eight intromissions) paired with the initially non-preferred or control chambers respectively, as in the previous experiment. However in this final experiment, the conditioning phase was extended to six conditioning days, during which males received alternate pairings of ejaculation or intromissions (3 of each pairing). Half of the males received an ejaculation pairing on the first conditioning day, and half received an intromissions pairing.

Data analysis

The pretest and posttest data collected from the sexual behavior CPP experiments were expressed as the preference score (the percentage of time spent in the sex-paired chamber) and the difference score (time spent in the sex-paired chamber minus time spent in the non sex-paired chamber). Paired t-tests were used to analyze the significance of the preference score and the difference score between the pretest and the posttest. In addition, a Pearson Product Moment Correlation test was used to analyze a possible correlation between the numbers of intromissions and the posttest preference score and difference score within each experiment. The criterion for significance was set at 0.05.

Results

Results from the first experiment, showed that sexually naïve males acquire a significant CPP to the ejaculation-paired chamber, as shown by a comparison of time spent in the chambers during the pretest and posttest (Fig. 1). Both the preference score ($p=0.023$) and the difference score ($p=0.012$) showed a significant increase in time spent in the ejaculation-paired chamber. In addition, sexually naïve males also acquired a significant CPP to the intromission-paired chamber. After conditioning, naïve males spent significantly more time in the intromission-paired chamber than the control chamber ($p=0.006$ preference score; $p=0.005$ difference score; Fig. 1). Un-stimulated control males did not form any preference (Preference score: Pretest versus Posttest: $35.8\% \pm 2.9$ versus $38.3\% \pm 2.7$, $p=0.47$; Difference score: Pretest versus Posttest: $168.4 \text{ sec} \pm 34.4$ versus $152.4 \text{ sec} \pm 33.3$, $p=0.71$).

Results from the second experiment revealed that males that received sexual experience prior to CPP testing also formed a CPP to the ejaculation-paired chamber, as shown by a significant increase in both preference score ($p<0.001$) and difference score ($p<0.001$; Fig. 2). However, in contrast to the findings in sexually naïve males, sexually experienced males did not form a CPP to the intromissions-paired chamber. Neither the preference score ($p=0.183$) nor the difference score ($p=0.235$) significantly changed after conditioning (Fig. 2).

In the third and fourth experiments, the hypothesis that ejaculation is more rewarding compared to intromissions was tested. The results of these studies showed, first, males that were sexually naïve prior to CPP testing did not form a CPP to the ejaculation-paired chamber relative to the intromissions-paired chamber after only one pairing of the respective sex behavior with the chamber (Fig. 3). We hypothesized that a single pairing of ejaculation and intromission was not sufficient to induce a difference in formation of CPP in sexually naïve animals. Therefore, a fourth experiment was conducted with an extended conditioning period consisting of three of each type of conditioning trial. Indeed, after three pairings each of intromissions and ejaculation, males showed a significant increase in both the preference and difference scores ($p<0.001$ for preference and difference scores; Fig. 3) for the ejaculation-paired relative to the intromission-paired chamber. Thus, with multiple pairings, ejaculation induced the formation of a CPP when compared to display of intromissions without ejaculation.

In each of the experiments, when pairings were performed with display of intromissions, males were allowed to display 6–8 intromissions, since this closely matches the number of intromissions normally preceding ejaculation (Coolen et al., 1996; Coolen et al., 2003a). Indeed, many males in the ejaculation-paired groups displayed 8 or fewer intromissions prior to ejaculation. However, some males in each of the experiments displayed more than 8 intromissions prior to ejaculation. Therefore, to rule out a positive correlation between the numbers of intromissions and formation of CPP, a correlation analysis was performed. This analysis revealed there were no correlations in any of the experiments between the numbers of intromissions and expression of CPP.

Discussion

The current study tested the hypotheses that ejaculation has a greater rewarding value compared to display of intromissions when examined using the CPP paradigm and that sexual experience influences the rewarding properties of intromissions. Indeed, it was demonstrated that ejaculation, but not intromissions resulted in the acquisition of CPP in sexually experienced animals. Moreover, sexually naïve males acquired a CPP for an ejaculation-paired environment over an intromission-paired environment. Sexual experience affected the rewarding value of intromissions (without ejaculation), since the display of ejaculation was only found to be essential for CPP in sexually experienced males, as sexually naïve males developed CPP following intromissions as well as ejaculation.

CPP is a well-established paradigm used to study the rewarding properties of sexual behavior (Hughes et al., 1990; Mehrara and Baum, 1990; Miller and Baum, 1987). Two variations in the CPP procedure, postcopulatory CPP and copulatory CPP, differ in whether the mating takes place in the CPP chamber or not (Pfaus et al., 2001). In the first procedure, which was used in the current study, male rats are allowed to copulate in a separate arena and then transferred immediately to one distinctive compartment of the CPP apparatus. In the second procedure, copulation to ejaculation is allowed to occur within the CPP chamber itself. Both procedures result in robust and reliable CPP. However, the postcopulatory CPP was used in the present study to eliminate a possible influence of anticipation of sexual reward on the formation of CPP. When male rats are exposed to environmental cues that are associated with prior sexual behavior, the mesolimbic system becomes activated (Balfour et al., 2004) presumably reflecting anticipation in response to the conditioned cues. The use of a copulatory CPP paradigm will therefore lead to influences of exposure to conditioned cues associated with the prior sexual experience in the CPP chamber. Hence, in order to isolate the role of intromissions and ejaculation in sexually naïve and experienced animals on formation of CPP, the postcopulatory procedure was used.

Another variable in CPP experiments is the number of conditioning trials: Either single (Straiko et al., 2007) or multiple pairings (Garcia Horsman and Paredes, 2004; Hughes et al., 1990; Miller and Baum, 1987) result in mating-induced CPP. Since one objective of the current study was to investigate the influence of sexual experience on sex-induced CPP, single pairings were utilized for the majority of the experiments in order to prevent reaching a ceiling of mating-induced CPP. Indeed, our results confirmed previous studies (Agmo and Berenfeld, 1990) that a single pairing of ejaculation to an environment, is sufficient for the acquisition of CPP in males.

In the present experiments, sexually naïve males acquired a CPP for an ejaculation-paired environment over an intromission-paired environment indicating that ejaculation has a greater reward value than intromissions. This result is consistent with previous learning/reinforcement studies in T-mazes (Kagan, 1955), straight-arm runway (Lopez et al., 1955), or hurdle climbing (Sheffield et al., 1951), which show that ejaculation is more reinforcing than mounts or intromissions alone. In addition, ejaculation is essential for the formation of conditioned copulatory preferences (Kippin and Pfaus, 2001). Together, these findings support the hypothesis put forward by Crawford et. al. (1993) that “strength of sex as a reinforcer is directly related to the extent to which subjects complete the copulatory behavioral sequence”.

In the present experiment, the preference for ejaculation-associated environment over intromission-paired chamber required multiple conditioning trials, and a single pairing was not sufficient to induce CPP. One explanation is that in sexually naïve animals, one pairing of each is not enough to differentiate the rewarding value of ejaculation and intromissions. Another explanation may be that the rewarding properties of intromissions reduce with sexual

experience, consistent with the finding that ejaculation is essential for CPP in sexually experienced males while intromissions induce CPP in sexually naive animals.

The finding that ejaculation has greater rewarding properties than intromissions supports the hypothesis that separate neural pathways exist for processing of ejaculation- or intromission-related signals. In support of this hypothesis, several studies in rat, hamster, and gerbil using Fos as a marker for neural activation have demonstrated neural activation specifically induced by ejaculation, but not by mounts or intromissions, in small subregions of the medial amygdala, bed nucleus of the stria terminalis, preoptic area, and posterior intralaminar thalamus (Coolen et al., 1996; Coolen et al., 1997; Coolen et al., 2003a; Heeb and Yahr, 1996; Kollack-Walker and Newman, 1997). We recently tested if these ejaculation-activated areas receive inputs from the lumbosacral spinal cord, and demonstrated using retrograde tracing and hemisections that the ejaculation-activated subregion of the posterior intralaminar thalamus, i.e. medial subparafascicular thalamic nucleus (mSPFp) receives a unique input from a population of neurons in the lumbar spinal cord (Coolen et al., 2003a; Coolen et al., 2003b; Ju et al., 1987; Truitt et al., 2003b). In turn these cells express Fos specifically with ejaculation, but not with mounts or intromissions, in support of their role in processing ejaculation-related cues (Truitt et al., 2003a). Moreover, this population of lumbar mSPFp-projecting cells are in the anatomical position to receive ejaculation-related sensory inputs from peripheral organs and express numerous neuropeptides, including galanin, cholecystokinin, and the opioid enkephalin (Coolen et al., 2003b; Ju et al., 1987; Nicholas et al., 1999). Currently, it is unknown if this pathway and the neurotransmitters within, functionally contributes to ejaculation-induced behaviors, such as CPP.

In summary, the present results demonstrate that ejaculation is the most rewarding component of male sexual behavior in rats. Moreover, we conclude that sexual experience influences the rewarding properties of the components of sexual behavior, i.e. intromissions, as intromissions induce CPP in sexually naïve, but not in sexually experienced males.

Acknowledgements

This research was supported by grants from the National Institutes of Health (R01 DA014591) and Canadian Institutes of Health Research to LMC.

References

- Agmo A, Berenfeld R. Reinforcing properties of ejaculation in the male rat: role of opioids and dopamine. *Behav Neurosci* 1990;104:177–182. [PubMed: 2156520]
- Agmo A, Gomez M. Sexual reinforcement is blocked by infusion of naloxone into the medial preoptic area. *Behav Neurosci* 1993;107:812–818. [PubMed: 8280390]
- Balfour ME, Yu L, Coolen LM. Sexual behavior and sex-associated environmental cues activate the mesolimbic system in male rats. *Neuropsychopharmacology* 2004;29:718–730. [PubMed: 14694350]
- Carr, GD.; Fibiger, HC.; Phillips, AG. Conditioned place preference as a measure of drug reward. In: Liebman, JM.; Cooper, SJ., editors. *The neuropharmacological basis of reward*. Oxford University Press; New York: 1989. p. 264-319.
- Coolen LM, Allard J, Truitt WA, McKenna KE. Central regulation of ejaculation. *Physiol Behav* 2004;83:203–215. [PubMed: 15488540]
- Coolen LM, Olivier B, Peters HJ, Veening JG. Demonstration of ejaculation-induced neural activity in the male rat brain using 5-HT1A agonist 8-OH-DPAT. *Physiol Behav* 1997;62:881–891. [PubMed: 9284512]
- Coolen LM, Peters HJ, Veening JG. Fos immunoreactivity in the rat brain following consummatory elements of sexual behavior: a sex comparison. *Brain Res* 1996;738:67–82. [PubMed: 8949929]

- Coolen LM, Veening JG, Petersen DW, Shipley MT. Parvocellular subparafascicular thalamic nucleus in the rat: anatomical and functional compartmentalization. *J Comp Neurol* 2003a;463:117–131. [PubMed: 12815751]
- Coolen LM, Veening JG, Wells AB, Shipley MT. Afferent connections of the parvocellular subparafascicular thalamic nucleus in the rat: evidence for functional subdivisions. *J Comp Neurol* 2003b;463:132–156. [PubMed: 12815752]
- Garcia Horsman P, Paredes RG. Dopamine antagonists do not block conditioned place preference induced by paced mating behavior in female rats. *Behav Neurosci* 2004;118:356–364. [PubMed: 15113261]
- Heeb MM, Yahr P. c-Fos immunoreactivity in the sexually dimorphic area of the hypothalamus and related brain regions of male gerbils after exposure to sex-related stimuli or performance of specific sexual behaviors. *Neuroscience* 1996;72:1049–1071. [PubMed: 8735229]
- Hughes AM, Everitt BJ, Herbert J. Comparative effects of preoptic area infusions of opioid peptides, lesions and castration on sexual behaviour in male rats: studies of instrumental behaviour, conditioned place preference and partner preference. *Psychopharmacology (Berl)* 1990;102:243–256. [PubMed: 2274607]
- Ju G, Melander T, Ceccatelli S, Hokfelt T, Frey P. Immunohistochemical evidence for a spinothalamic pathway co-containing cholecystokinin- and galanin-like immunoreactivities in the rat. *Neuroscience* 1987;20:439–456. [PubMed: 2438590]
- Kagan J. Differential reward value of incomplete and complete sexual behavior. *J Comp Physiol Psychol* 1955;48:59–64. [PubMed: 14354074]
- Kippin TE, Pfau JG. The development of olfactory conditioned ejaculatory preferences in the male rat. I. Nature of the unconditioned stimulus. *Physiol Behav* 2001;73:457–469. [PubMed: 11495649]
- Kollack-Walker S, Newman SW. Mating-induced expression of c-fos in the male Syrian hamster brain: role of experience, pheromones, and ejaculations. *J Neurobiol* 1997;32:481–501. [PubMed: 9110260]
- Lopez HH, Olster DH, Ettenberg A. Sexual motivation in the male rat: the role of primary incentives and copulatory experience. *Comp Physiol Psychol* 1955;48:59–64.
- Martinez I, Paredes RG. Only self-paced mating is rewarding in rats of both sexes. *Horm Behav* 2001;40:510–517. [PubMed: 11716580]
- Mehrara BJ, Baum MJ. Naloxone disrupts the expression but not the acquisition by male rats of a conditioned place preference response for an oestrous female. *Psychopharmacology (Berl)* 1990;101:118–125. [PubMed: 2343072]
- Miller RL, Baum MJ. Naloxone inhibits mating and conditioned place preference for an estrous female in male rats soon after castration. *Pharmacol Biochem Behav* 1987;26:781–789. [PubMed: 3602035]
- Nicholas AP, Zhang X, Hokfelt T. An immunohistochemical investigation of the opioid cell column in lamina X of the male rat lumbosacral spinal cord. *Neurosci Lett* 1999;270:9–12. [PubMed: 10454133]
- Pfau JG, Kippin TE, Centeno S. Conditioning and sexual behavior: a review. *Horm Behav* 2001;40:291–321. [PubMed: 11534994]
- Pfau JG, Phillips AG. Role of dopamine in anticipatory and consummatory aspects of sexual behavior in the male rat. *Behav Neurosci* 1991;105:727–743. [PubMed: 1840012]
- Sheffield FD, Wulff JJ, Backer R. Reward value of copulation without sex drive reduction. *J Comp Physiol Psychol* 1951;44:3–8. [PubMed: 14814235]
- Straiko MM, Gudelsky GA, Coolen LM. Treatment with a serotonin-depleting regimen of MDMA prevents conditioned place preference to sex in male rats. *Behav Neurosci* 2007;121:586–593. [PubMed: 17592950]
- Truitt WA, Domingues JM, Adelman J, Coolen LM. Galanin infusions into the medial parvocellular parafascicular thalamic nucleus inhibit male sexual behavior. *Horm Behav* 2003a;44:81.
- Truitt WA, Shipley MT, Veening JG, Coolen LM. Activation of a subset of lumbar spinothalamic neurons after copulatory behavior in male but not female rats. *J Neurosci* 2003b;23:325–331. [PubMed: 12514231]

Figure 1.

One pairing with display of ejaculation (A, B) or intromissions (C, D) induced CPP in sexually naive males. (A, C) preference score, the percentage of time spent in the ejaculation-(A) or intromissions- (C) paired chamber. (B, D) difference score, time (in seconds) spent in the ejaculation- (B) or intromissions- (D) paired chamber minus time spent in the control chamber. * $p < 0.05$ compared to pretest.

Figure 2.

One pairing with display of ejaculation (A, C), but not with intromissions (C, D) induced CPP in sexually experienced males. (A, C) preference score, the percentage of time spent in the ejaculation- (A) or intromissions- (C) paired chamber. (B, D) difference score, time (in seconds) spent in the ejaculation- (B) or intromissions- (D) paired chamber minus time spent in the control chamber. * $p < 0.05$ compared to pretest.

Figure 3.

One pairing with display of ejaculation did not induce CPP in sexually naïve males when the control chamber was paired with display of intromissions (A, B). In contrast, males that were sexually naïve prior to onset of conditioning acquired CPP for the ejaculation paired chamber over the intromissions paired control chamber after three pairings (C, D). (A, C) preference score, the percentage of time spent in the ejaculation-paired chamber. (B, D) difference score, time in seconds spent in the ejaculation-paired chamber minus time spent in the intromissions-paired chamber. * $p < 0.05$ compared to pretest.

Table 1

Conditioning design and group numbers for each of the four sexual behavior experiments

Experiment	Sexual Experience of Males	Conditioning Trials	Sex-Pairing	Control- Pairing
1	Naïve	1	Ejaculation (n=10) OR Intromissions only (n=11)	No sex behavior
2	Experienced	1	Ejaculation (n=10) OR Intromissions only (n=10)	No sex behavior
3	Naïve	1	Ejaculation (n=8)	Intromissions only
4	Naïve	3	Ejaculation (n=10)	Intromissions only