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Non-intromissive mating stimuli are sufficient to enhance sexual behaviors in ovariectomized female rats

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Abstract

When ovariectomized/adrenalectomized female rats, injected with subthreshold doses of estradiol are given copulatory stimulation by a male rat at half hour intervals, the level of lordosis gradually increases over the course of a few hours. We tested the hypothesis that paracopulatory behaviors (behaviors that occur repetitively prior to and between mounts), also generally considered to be heavily dependent on progesterone, are enhanced by this stimulation as well. We have reported previously that the enhancement of copulatory behavior is dependent to a large extent on intromissive stimulation by the male. In the present study, mating stimulation induced high levels of paracopulatory behaviors, as well as lordosis. Surprisingly, though, and in contrast to previous findings, this increase was seen not only in rats receiving intromissive stimulation, but in those receiving non-intromissive stimulation as well. Furthermore, intromissive stimulation induced high levels of rejection behavior. In a subsequent experiment, experimenter-induced, mechanical stimulation increased only rejection behaviors, not copulatory behavior. The results collectively demonstrate that, under the conditions used in these experiments, non-intromissive stimulation is sufficient for inducing both copulatory and paracopulatory behaviors in estradiol-primed rats. However, under the conditions used in these studies, intromissive stimulation increases rejection behaviors.

Keywords

Lordosis; Sexual receptivity; Copulatory behavior; Paracopulatory behaviors; Proceptive behaviors; intromission; Vaginal stimulation

Introduction

The ovarian hormones, estradiol and progesterone, have predictable influences on the expression of feminine sexual behavior in female rats. Ovariectomy eliminates the expression of sexual behaviors in rats by the loss of cyclic release of estradiol and progesterone (Boling and Blandau, 1939; Powers, 1970). Although ovariectomized (OVX) rats may respond to estradiol alone, treatment with progesterone typically increases the effectiveness of estradiol (Whalen, 1974), makes the period of sexual receptivity more discrete, and increases paracopulatory (Erskine, 1989; Tennent et al., 1980) (also called

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proceptive) behaviors (we have used the term “paracopulatory” (Blaustein and Erskine, 2002) for those behaviors that occur repetitively prior to and between mounts in order to obviate the assumptions about the female's motivation inherent in the terms “proceptive” and “solicitational”). After heat terminates, animals become refractory to facilitation of sexual behavior by progesterone (Blaustein and Erskine, 2002; Blaustein and Olster, 1989; Feder and Marrone, 1977; Morin, 1977). As estradiol and progesterone have reliable influences on feminine sexual behavior in rats, this provides a physiologically-relevant model with which to investigate the cellular processes by which estradiol and progesterone act on the brain, as well as other influences on these cellular processes.

Sexual receptivity is regulated not only by steroid hormones, but also by afferent input from mating stimulation. This includes vaginocervical stimulation (VCS) associated with intromissions and ejaculations by the male. Some of these changes are short latency, and some are of longer latency. For example, when females have the opportunity to escape, they take longer to return to the male after an intromission or an ejaculation than after a mount (Bermant, 1961; Bermant and Westbrook, 1966; Erskine, 1985). Thus, mounts that include VCS result in a short-term inhibition of sexual responsiveness. The presence of VCS also results in a greater intensity of the lordosis reflex (Diakow, 1975), and VCS administered by a glass probe along with manual palpation of the flanks and perineum intensifies the lordosis response (Komisaruk, 1971; Rodriguez-Sierra et al., 1975).

Facilitation of the expression of lordosis over the course of a few hours is seen in females exposed to sexually active males over successive mating tests (Foreman and Moss, 1977; Rajendren et al., 1990; Rajendren and Moss, 1993). This behavioral enhancement is not dependent upon the presence of gonadal or adrenal progesterone (Auger et al., 1997; Foreman and Moss, 1977). We have reported that intromissive stimulation is necessary and sufficient for this potentiation of lordosis (Bennett et al., 2001); elimination of intromissive stimulation by placing tape over the vagina blocked much of the enhancement, while experimenter-induced VCS with a plastic probe slightly enhanced subsequent response to males, albeit not to the level seen when male rats provide the stimulation. Although, in other cases, sexual receptivity also increases over the course of extended testing (Clemens et al., 1969; Hardy and Debold, 1973; Larsson et al., 1974), adrenalectomy prevents that increase (Larsson et al., 1974), and mere handling can substitute for mating (Hardy and Debold, 1973). Therefore, this increase seems to depend on the release of progesterone from the adrenal gland, and it does not seem to be due to direct afferent input from the genital region to the brain.

In earlier work, we have reported results of mating stimulation only on lordosis. We have shown that the induction of the expression of lordosis by mating is dependent on ligand-independent activation of progestin receptors (Auger et al., 1997). If mating stimulation activates progestin receptors by ligand independent activation, and if this activation of progestin receptors has similar neuronal effects to activation by progesterone, then we predicted that paracopulatory behaviors, such as ear-wiggling and darting-and-hopping, believed to be heavily dependent on progestins, would increase as well. In Experiment 1, we attempted to extend the results of our earlier work by determining the effects of intromissive stimulation on paracopulatory behaviors (*e.g.*, ear-wiggling) (Blaustein and Erskine, 2002). Although the results of Experiment 1 suggested that mating stimulation was sufficient to induce paracopulatory behavior, as well as lordosis, we obtained the surprising result that intromissive stimulation was not essential for either. Because this finding conflicted with our previous report that intromissive stimulation was necessary for enhancement of lordosis, this finding was followed up in two subsequent experiments.

Experiment 1: Mating enhancement of sexual behaviors

In the absence of progesterone, mating stimulation increases the expression of sexual receptivity in estradiol-primed, ovariectomized–adrenalectomized female rats (Auger et al., 1997). This enhancement of sexual receptivity by mating is believed to be facilitated through ligand-independent activation of progestin receptors. Because paracopulatory behaviors, like ear-wiggling, are considered to be dependent on progesterone, we determined if a paracopulatory behavior is enhanced by repeated mating stimulation, as is lordosis. We also determined if the effects of repeated mating stimulation on rejection behavior.

Methods

Twenty-six female CD rats weighing 175–200 g were obtained from Charles River Breeding, Wilmington, MA). They were group-housed, maintained on a 14:10 reversed L:D cycle, and given *ad lib* access to food (Teklad Phytoestrogen reduced Lab diet # 2014) and water.

Surgery

In order to remove all peripheral sources of progesterone, one week after arrival in the laboratory, rats were bilaterally ovariectomized and adrenalectomized under ketamine cocktail anesthesia (26 mg/kg ketamine hydrochloride, 5 mg/kg xylazine hydrochloride, and 0.9 mg/kg acepromazine), and implanted subcutaneously in the nape of the neck with a corticosterone pellet, a mixture of corticosterone (15%; Steraloids, Inc., Newport, RI) and cholesterol (85%; Steraloids, Inc.), weighing approximately 100 mg (prepared as described by Akana et al. (1985)). Rats were provided with salt (sodium chloride) spools in their cages immediately following surgery and throughout the course of the experiment. Rats were handled daily starting from the day of surgery onward. All procedures were pre-approved by the Institutional Animal Care and Use Committee at the University of Massachusetts, Amherst. For timeline of experiment, refer to Fig. 1.

One week after surgical removal of the ovaries and adrenal glands, rats were screened for sexual receptivity following subcutaneous injection of 5 µg estradiol benzoate (EB; dissolved in 0.1 ml sesame oil). Forty-four hours after EB injections, rats were tested for five mounts for the presence of sexual behavior (lordosis quotient (LQ) defined as the number of lordosis responses divided by the number of mounts by the male, multiplied by 100) in the absence of progesterone. Sexually vigorous Long–Evans male rats were used for stimulus animals. They were then injected with 500 µg progesterone (dissolved in 0.1 ml sesame oil containing 5% benzyl alcohol and 15% benzyl benzoate w/v). Four hours later, the rats were tested for sexual receptivity and their level of sexual receptivity was assessed. Rats were considered sexually receptive if they had an LQ greater than 60. One female responded poorly to this hormonal regimen and was subsequently excluded from the experiments. All behavioral tests took place during the dark phase of the light/dark cycle in cylindrical polycarbonate arenas using sexually vigorous males.

Repeated mating procedure

One week after screening, rats were injected with 5 µg estradiol benzoate. Forty four hours later they were repetitively tested with a sexually vigorous male rat to test for enhancement of sexual behavior. In one group, the vagina of each rat was covered with a small piece of duct tape (1 in.×1 in.) before each mating test to prevent intromissions (masked), and the other group was without tape (unmasked). All behavioral testing was done under dim red light during the dark phase of the light/dark cycle.

Every 30 min, females were placed in a circular polycarbonate arena ($d = 50$ cm, $h = 40$ cm) with a sexually vigorous male until she received 10 mounts. This continued for a total of ten tests (*i.e.*, total of 5 h). Females were returned to their home cages between tests. Receptive behavior (lordosis), a paracopulatory behavior (bouts of ear-wiggling) (Blaustein and Erskine, 2002) and rejection behaviors (facing-off, boxing and kicking of the male by the female when the male attempted to mount) were recorded. Lordosis intensity was defined as the average intensity of spinal dorsiflexion based on a four point scale of 0–3 (Hardy and DeBold, 1972) and calculated as the mean of those mounts which elicited a lordosis response. Individual scores of zero were not included in the calculation of the mean lordosis intensity of the group. It should be noted that this differs from an earlier term related to intensity of lordosis, lordosis rating, and is being used as an indicator of intensity of lordosis only on those mounts that resulted in a lordosis response. Paracopulatory and rejection behaviors were quantified by counting the total number of their occurrences during each session for each subject. In order to eliminate animals responding to estradiol alone with the expression of sexual receptivity, only females with LQs of 30 or less during the first mating session were included in the study. This resulted in the elimination of five rats, leaving an N of 21. The group size for the masked animals was 11, and the size of the unmasked group was 10.

One week later, all rats were injected with EB followed by progesterone 44 h later. One week after this treatment, they were again injected with EB using the same procedure for mating enhancement already described, except that treatment was reversed, so that each female was tested only once under each condition.

Statistical tests

All behavioral measures were analyzed by two way repeated measures analysis of variance (ANOVA) using SigmaStat Statistical Analysis System 3.0. Student–Newman–Keuls *post hoc* analysis was used to test for differences if significant *F* values were found ($p < 0.05$).

Results

As reported previously, the LQ in the unmasked group increased steadily during the first few hours of testing (Fig. 2). Surprisingly, and in contrast to earlier work from this laboratory, the masked and unmasked groups each increased in their LQ over the course of ten mating tests ($F(9, 171) = 33.647, p < .001$), and there was no statistically significant effect of treatment ($F(1, 171) = 1.100, p > .05$) (Fig. 2A). There also was no significant effect of treatment ($p > .05$) or test number on lordosis intensity (Fig. 2B). Likewise, both masked females and unmasked females increased the amount of the paracopulatory behavior, ear-wiggling, over the course of testing ($F(9, 171) = 7.659, p < .001$) (Fig. 2C), and there was no statistically significant difference between the two treatments ($F(1, 171) = .0329, p > .05$) (Fig. 2D).

In contrast, prevention of intromissive stimuli by vaginal masking had a dramatic effect on rejection behavior. Unmasked females showed significantly higher levels of rejection behavior in all mating enhancement tests from the third on, contrasted with masked females ($F(1, 171) = 11.384, p < .005$), and there was a statistically significant treatment $\times$ test number interaction ($F(9, 171) = 2.182, p < .05$).

Experiment 2: Mating enhancement of sexual behaviors

The surprising result of Experiment 1 that both masked and unmasked females increased lordosis and ear-wiggling as a consequence of mating stimulation conflicts with our earlier report in which we observed that masking prevented the enhancement of lordosis by mating stimulation (Bennett et al., 2001). The results support the idea that mating stimulation,

regardless of whether there is accompanying intromissive stimulation, induces both lordosis and a paracopulatory behavior (ear-wiggling), as is the case with progesterone facilitation of sexual behavior. Therefore the non-intromissive sensory stimuli obtained from the male seem to be sufficient to induce both receptive and paracopulatory behaviors, but intromissive stimuli have a negative effect by increasing rejection toward the male. Because the results of Experiment 1 conflict with our earlier published work (Bennett et al., 2001), this experiment was replicated with similar methods.

Methods

The methods were the same as Experiment 1, except that a dose of 2 µg EB was used for the screening test. During the first mating session, four females from the masked group and three females from the unmasked group exhibited an LQ higher than 30. These females were subsequently eliminated from the study, because it was assumed that they were responding to estradiol alone. The final size of masked and unmasked groups were $n = 9$ and $n = 8$ respectively.

Each female was injected subcutaneously with 5 µg EB and 44 h later placed in a testing arena with a sexually active male rat for up to 15 min until ten mounts were received every half hour for a total of 5 h. In addition to ear-wiggling, instances of darting-and-hopping were recorded.

Results

As in Experiment 1, mating stimulation with or without intromissive stimulation induced high levels of lordosis over the 5 h of testing ($F(9, 135) = 34.44, p < 0.001$) (Fig. 3A). However there was no significant effect of treatment ($F(1, 135) = .740, p > .05$), nor a treatment $\times$ test interaction ($F(9, 135) = 0.901, p > .05$). Likewise, no significant difference was detected between the two groups in lordosis intensity ($F(1, 9) = 2.56, p = 0.126$) (Fig. 3B).

Hops and darts, ear wiggling and rejection showed a fairly high degree of variability in this experiment. Ear-wiggling increased significantly ($F(9, 135) = 11.219, p < .001$) (Fig. 3C). Although there was no effect of treatment, there was a statistically significant treatment $\times$ test interaction ($F(9, 135) = 2.73, p < 0.01$), suggesting that ear wiggling increased more in the masked group. Likewise hops and darts increased significantly ($F(3, 51) = 8.32, p < .001$ by Huyn-Feldt correction), although there was a great deal of variability among animals (Fig. 3D). However, there was no significant treatment $\times$ test interaction by Huyn-Feldt correction ($F(3, 51) = 2.49, p > .05$). Rejection behavior was also much more variable in this study than in Experiment 1 (Fig. 3E). Although there was a tendency for unmasked rats to show higher levels of rejection throughout the tests, the data failed the test for normality.

Experiment 3: Influence of experimenter-applied VCS on sexual behavior

Because of the unexpected results of Experiment 1 and its confirmation in Experiment 2, we investigated the effects of manual VCS on subsequent lordosis. In our earlier report, we found that experimenter-applied, mechanical VCS induces a small, but statistically significant increase in lordosis in estradiol-primed rats 1 h after stimulation (Bennett et al., 2001).

Methods

Fifteen female rats were ovariectomized, and they were then handled daily. One week later, they were injected with 2 µg EB, followed 48 h later with 500 µg progesterone. Four hours later, they were screened for the presence of sexual behaviors with a sexually active male

rat. Ten days after screening, females were injected with 2 μ g EB. Forty-eight hours later, they were placed with a sexually active male rat until three mounts were received in order to assess the presence of estradiol-only induced sexual receptivity. Rats expressing an LQ of 60 or less during the first pretest or any lordosis responses during the second screening pretest were removed from the experiment. The remaining 14 rats were placed in a clean testing arena and administered VCS with a plastic probe as described earlier (Bennett et al., 2001) or administered scapular stimulation. In the first part of this experiment, rats were injected with 2 μ g EB. Forty-four hours later, they received 300 g of pressure (2 s every 30 s for 15 min followed by 15 min with no stimulation followed by repeating the 15 min of intermittent stimulation) with a 1 cm³ plastic syringe plunger attached to a force gauge. Stimulation was applied either to the cervix or to the scapular region (controls). Ninety minutes after the start of treatment, each female's vagina was covered as described in Experiment 1, and the female was placed in a testing arena with a sexually active male until ten mounts were received, and incidences of lordosis, paracopulatory behaviors (darting and hopping and ear-wiggling), and rejection behaviors were recorded as described in Experiments 1 and 2 with the experimenter blind to treatment groups. At the end of the session, all females were injected with 500 μ g progesterone, and they were then retested 4 h later for the presence of sexual receptivity. All animals responded to progesterone with lordosis responses.

In the second phase of this study, 15 rats were treated identically, except that they received 150 g of pressure to determine the effects of lighter pressure. Ninety minutes after the start of treatment, they were tested for lordosis with a sexually active male as in the first part of this experiment. Beginning one week after the experiment, they were injected with EB and rescreened for progesterone-independent sexual behavior, and the experiment was repeated with the treatments reversed. One animal was removed from the experiment because she showed lordosis responses in the EB-only screening test.

Results

The application of 300 g of pressure was without significant effect on LQ (Fig. 4 top panel). Because the data failed the normality test, the Mann–Whitney Rank Sum Test was used ($T(7,7) = 66.50, p > .05$). However, VCS induced a statistically significant increase in rejection behaviors ($t(12) = 2.80, p < .02$). No paracopulatory behaviors were observed in either group. Likewise, 150 g of pressure did not result in a significant effect on lordosis ($t(27) = .50, p > .05$, but as with 300 g, it induced a high level of rejection ($t(27) = 2.10, p < .05$) (Fig. 4 bottom panel). No paracopulatory behaviors were observed in either group.

Discussion

The results of Experiment 1 demonstrate that copulatory stimulation induces the paracopulatory behavior, ear-wiggling, as well as the previously reported lordosis in repeatedly mated, estradiol-primed, OVX–ADX female rats. However, contrary to our previous report (Bennett et al., 2001), non-intromissive mating stimulation was sufficient to enhance sexual behavior; the female rats expressed high levels of sexual behaviors following repeated mating with intromissive stimulation prevented by the placing of tape over the vagina. Interestingly, there was a strong effect of preventing intromissive stimulation by vaginal masking on rejection behavior; preventing intromissive stimuli nearly completely suppressed the increase in rejection behavior seen in unmasked rats.

Because these results were unanticipated, a second experiment using similar procedures was conducted. The results were reasonably consistent between the two experiments. Furthermore an increase in a second paracopulatory behavior, hops and darts, was also observed. Although there was a tendency for a higher level of rejection behaviors in the

unmasked group, due to the greater variability in the unmasked group in the second experiment contrasted with the first, this was not statistically significant. These experiments are critical to an understanding of the processes by which genitosensory stimulation can enhance sexual behaviors. The finding that non-intromissive stimulation is sufficient, and VCS is not necessary, has major implications for the neural pathways involved in this response.

These results also have relevance to a recently proposed model for the neural regulation of sexual behaviors (Pfaff et al., 2008). This model predicts that treatment of the periaqueductal gray, unlike the hypothalamus, may be able to dissociate effects on lordosis and defensive behaviors (*i.e.*, rejection). In the present work, intromissive stimulation increases both, lordosis and rejection behaviors, whereas non-intromissive stimulation increases only lordosis. The mating enhancement procedures used in this paper would allow tests of some of the predictions of this model.

Although we do not have a definitive explanation for the discrepancy between the earlier report from this laboratory (Bennett et al., 2001) in which enhancement of sexual behavior was blocked by vaginal masks and the present results, there were some procedural differences between the two studies that may have contributed to the difference. Bennett et al. (2001) did not provide corticosterone replacement to compensate for the loss in this hormone after adrenalectomy, the doses of hormone were slightly different (5 μ g in the present work vs. 2 μ g in the earlier paper), and Long–Evans male rats were used in the present work as opposed to Sprague–Dawley rats in the earlier work. Subjectively, Long–Evans rats are more vigorous than Sprague–Dawley rats, so they might have provided a stronger stimulus. Regardless of the source for the discrepancy, the data show quite clearly that non-intromissive stimuli are sufficient under these conditions to enhance sexual behavior in the absence of progesterone. Furthermore, the present results are consistent with the idea that, because intromission is not physically possible in a female who is not displaying lordosis, the enhancement seen in response to mounts by a male rat virtually has to be due to non-intromissive stimuli.

In order to rule out the contribution of adrenal progesterone in the enhancement of sexual behavior, it was necessary to remove the adrenal glands in Experiments 1 and 2 because, like the ovaries, they can release sufficient levels of progesterone to facilitate sexual behavior in some circumstances (Feder and Ruf, 1969; Franck, 1977; Gorzalka and Moe, 1994). As mentioned earlier, in the previous study, OVX–ADX rats were given access to salt spoons to replace electrolytes, while in the present experiment, they were also given corticosterone replacement *via* a pellet releasing corticosterone at a constant rate. It is clear that neither situation fully compensates for the removal of the adrenal and duplicates the physiology of the intact rat. It should be noted that adrenalectomy may enhance the hormonal induction of sexual behavior (Eriksson and Sodersten, 1973; Gray and Gorzalka, 1980), and administration of corticosterone may suppress expression of sexual behavior (DeCatanzaro et al., 1981). Likewise, although the mediating factors are not known, removal of the adrenal gland increases sensitivity for low levels of mating stimulation (*i.e.*, fewer intromissions) to induce Fos expression in some forebrain regions (Cameron and Erskine, 2003).

Although the findings of Experiment 3 are consistent with and extend those of Experiments 1 and 2, the failure of experimenter-induced VCS to induce sexual behavior is also at odds with our previous report (Bennett et al., 2001) as well as the earlier work of Rodriguez-Sierra et al. (1975). However, in our previous report, the degree of facilitation was quite small. In a number of pilot experiments, we have sometimes observed increases by VCS, and we have sometimes observed increases with only control stimulation. The data strongly

suggest that there is no consistent effect of manual, experimenter-applied VCS on enhancement of lordosis in estradiol-primed OVX–ADX rats. However, consistent with Experiments 1 and 2, VCS induced a consistent increase in rejection behavior, which has been reported earlier under very different conditions (Hardy and DeBold, 1972; Rodriguez-Sierra et al., 1975). OVX rats were used in this experiment, and OVX–ADX rats were used in our previous experiment. This difference in methods would have worked in favor of our observing an increase in lordosis, since there was the potential for release of progesterone from the adrenal glands, which can facilitate the expression of sexual behavior in estradiol-primed rats. Obviously, this was not a factor in this experiment, since an increase in lordosis was not observed, while an increase in rejection in response to VCS was seen.

Another report from this laboratory (Bennett et al., 2002) has bearing on the interpretation of these results. We assessed the effects of extensive mating stimulation, which included intromissive stimulation by the male, on heat termination. As expected, an extended bout of mating, which included intromissions and ejaculations abbreviated the period of sexual receptivity (*i.e.*, the expression of lordosis) relative to females that did not receive this stimulation. However, repeated testing at 4 hour intervals in the rats that did not receive the previous stimulation extended heat duration contrasted with rats tested only once at either six, ten or 14 h after the start of mating. These results support the idea that intromissive stimulation contributes to mating-induced estrous abbreviation (Boling and Blandau, 1939; Erskine, 1985; Lodder and Zeilmaker, 1976; Pfaus et al., 2000; Reading and Blaustein, 1984), but they also suggest that non-intromissive mating stimulation in the repeatedly tested, masked control group enhances lordosis responding. Thus maintenance of sexual receptivity depends on a complex interplay of those factors that bring about the termination of sexual responding and those that enhance it.

The receipt of intromissions and ejaculations results in subsequent abbreviation of the period of estrus not only in rats (Boling and Blandau, 1939; Erskine, 1985; Lodder and Zeilmaker, 1976; Pfaus et al., 2000; Reading and Blaustein, 1984), but also guinea pigs (Goldfoot and Goy, 1970; Roy et al., 1993), hamsters (Carter, 1972; Carter et al., 1976; Carter et al., 1973; Carter and Schein, 1971; Ramos and Debold, 1999), and gerbils (Carter et al., 1980). In rats, pacing of sexual contacts increases the inhibitory effect of intromissive stimulation on heat duration (Coopersmith et al., 1996; Erskine, 1985).

The increase in active rejection behaviors in females receiving intromissive stimulation is consistent with a variety of earlier studies (Hardy and DeBold, 1972; Rodriguez-Sierra et al., 1975). It is also consistent with studies which looked at passive rejection, the avoidance of the male by the female, in testing situations that allowed the female to passively avoid the male (Pfaus et al., 2000). Under these conditions, termination of the expression of lordosis is preceded by the presence of active and passive rejection behaviors. Administration of 50 applications of VCS with a glass probe increased the avoidance of the male (passive rejection), as well as active rejection 16 to 20 h later (Pfaus et al., 2000). Although others (VanderSchoot et al., 1992) failed to find effects of previous copulatory stimulation on heat duration, the study by Pfaus and colleagues suggests that these investigators may not have waited a sufficiently long period of time to test for abbreviation. Similarly, in partner preference tests of sexual motivation, females display a preference for a sexually active male over other stimulus animals (Meyerson and Lindstrom, 1973; Pfeifle and Edwards, 1983). However, the time that the female spends with a male stimulus animal is lengthened if the female is masked to prevent intromissions (Broekman et al., 1988). The active rejection in a forced mating, testing situation in which passive rejection could not take place in the present work bears resemblance to the passive rejection shown in these other tests of sexual motivation.

In many behavioral neuroendocrinology experiments, experimenter-induced VCS with a mechanical probe has been used as a substitute for intromissions by the male. This has historical precedent in work on the induction of the progestational state or pseudopregnancy (Everett, 1964), the process that results in the rescue of the corpus luteum during the estrous cycle. It is noteworthy that experimenter-induced VCS with a mechanical probe has quite similar effects on neuronal response in most neural areas to extensive mating with intromissions by a male rat (Pfaus et al., 1993; Tetel et al., 1993). However, to the best of our knowledge, it is not known if the penis contacts the cervix during intromission, so it is unclear if a mechanical probe is a valid substitute for intromissions by the male. It is possible that the cervix only receives pressure during and after ejaculation when the penile cup and/or the copulatory plug put pressure on it. Therefore, it is possible that the proximal stimulus received during intromission is not cervical stimulation, but rather intense vaginal stimulation, as the keratinous spines (Phoenix et al., 1976; Sachs et al., 1984) of the rat penis rake the vaginal wall. It should be noted that while the mechanical stimulus used here puts quantifiable pressure on the cervix, it exerts little pressure on the vagina, and certainly not the quality or quantity received by the female rat during intromission. Physiologically, it is possible that stimulation of the cervix and vagina each have some similar influences, since each is innervated by the pelvic nerve (Komisaruk et al., 1972; Peters et al., 1987). Because there is no obvious experimenter-induced stimulus that can substitute for the friction of the keratinous spines, VCS provided by a plastic or glass rod is the closest approximation, but caution should be exercised in interpretation of experiments in which it is used. The stimulation provided by VCS with a mechanical probe may have more in common with the stimulation received from ejaculation than from intromission.

In a recent report, Meerts and Clark (2009) reported that VCS administered with a technique similar to that used in Experiment 3, induces a conditioned place preference. This suggests that in some contexts, female rats find this type of VCS to be aversive, as shown by the increase in rejection to a male, and in other cases, it may serve as a stimulus sufficient to induce a conditioned place preference and therefore can be reinforcing. In the present experiment, only behavior toward a male in a circular arena was investigated, which is a very different context than the conditioned place preference apparatus used by Meerts and Clark.

In summary, the results of the present experiments demonstrate that mating enhancement of sexual behavior extends to paracopulatory behaviors, as well as lordosis. Furthermore, the results demonstrate conclusively that, under the circumstances used in these studies, intromissive stimulation is not essential for mating-enhancement of sexual receptivity. On the contrary, intromissive stimulation generally increases the incidence of the negative, rejection behaviors.

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References

- Akana SF, Cascio CS, Shinsako J, Dallman MF. Corticosterone: narrow range required for normal body and thymus weight and ACTH. *Am. J. Physiol.* 1985; 249:R527–R532. [PubMed: 2998210]
- Auger AP, Moffatt CA, Blaustein JD. Progesterone-independent activation of rat brain progesterin receptors by reproductive stimuli. *Endocrinology.* 1997; 138:511–514. [PubMed: 8977442]
- Bennett AL, Blasberg ME, Blaustein JD. Sensory cues mediating mating-induced potentiation of sexual receptivity in female rats. *Horm. Behav.* 2001; 40:77–83. [PubMed: 11467886]

- Bennett AL, Blasberg ME, Blaustein JD. Mating stimulation required for mating-induced estrous abbreviation in female rats: effects of repeated testing. *Horm. Behav.* 2002; 42:206–211. [PubMed: 12367573]
- Bermant G. Response latencies of female rats during sexual intercourse. *Science.* 1961; 133:1771–1773. [PubMed: 17829723]
- Bermant G, Westbrook WH. Peripheral factors in the regulation of sexual contact by female rats. *J. Comp. Physiol. Psychol.* 1966; 61:244–250. [PubMed: 5909304]
- Blaustein, JD.; Erskine, MS. Feminine sexual behavior: cellular integration of hormonal and afferent information in the rodent forebrain. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior*. New York: Academic Press; 2002. p. 139-214.
- Blaustein, JD.; Olster, DH. Gonadal steroid hormone receptors and social behaviors. In: Balthazart, J., editor. *Molecular and Cellular Basis of Social Behavior in Vertebrates*. Berlin: Springer-Verlag; 1989. p. 31-104.
- Boling JL, Blandau RJ. The estrogen–progesterone induction of mating responses in the spayed female rat. *Endocrinology.* 1939; 25:359–364.
- Broekman M, de Bruin M, Smeenk J, Slob AK, van der Schoot P. Partner preference behavior of estrous female rats affected by castration of tethered male incentives. *Horm. Behav.* 1988; 22:324–337. [PubMed: 3169698]
- Cameron N, Erskine MS. *c-fos* expression in the forebrain after mating in the female rat is altered by adrenalectomy. *Neuroendocrinology.* 2003; 77:305–313. [PubMed: 12806176]
- Carter CS. Postcopulatory sexual receptivity in the female hamster: the role of the ovary and adrenal. *Horm. Behav.* 1972; 3:261–265. [PubMed: 4681747]
- Carter CS, Getz LL, Gavish L, Mcdermott JL, Arnold P. Male-related pheromones and the activation of female reproduction in the prairie vole (*Microtus ochrogaster*). *Biol. Reprod.* 1980; 23:1038–1045. [PubMed: 7008851]
- Carter CS, Landauer MR, Tierney BM, Jones T. Regulation of female sexual behavior in the golden hamster: behavioral effects of mating and ovarian hormones. *J. Comp. Physiol. Psychol.* 1976; 90:839–850. [PubMed: 987076]
- Carter CS, Michael SJ, Morris AH. Hormonal induction of female sexual behavior in male and female hamsters. *Horm. Behav.* 1973; 4:129–141.
- Carter CS, Schein MW. Sexual receptivity and exhaustion in the female golden hamster. *Horm. Behav.* 1971; 2:191–200.
- Clemens LG, Hiroi M, Gorski RA. Induction and facilitation of female mating behavior in rats treated neonatally with low doses of testosterone propionate. *Endocrinology.* 1969; 84:1430–1438. [PubMed: 5769381]
- Coopersmith CB, Candurra C, Erskine MS. Effects of paced mating and intromissive stimulation on feminine sexual behavior and estrus termination in the cycling rat. *J. Comp. Psychol.* 1996; 110:176–186. [PubMed: 8681531]
- DeCatanzaro D, Knipping RP, Gorzalka BB. Antagonism of estrogen-induced lordosis by corticosterone in adrenalectomized–ovariectomized female rats and mice. *Pharmacol. Biochem. Behav.* 1981; 15:761–766. [PubMed: 7312911]
- Diakow C. Motion picture analysis of rat mating behavior. *J. Comp. Physiol. Psychol.* 1975; 88:704–712.
- Eriksson H, Sodersten P. A failure to facilitate lordosis behavior in adrenalectomized and gonadectomized estrogen-primed rats with monoamine-synthesis inhibitors. *Horm. Behav.* 1973; 4:89–97.
- Erskine MS. Effects of paced coital stimulation on estrous duration in intact cycling rats and ovariectomized and ovariectomized–adrenalectomized hormone-primed rats. *Behav. Neurosci.* 1985; 99:151–161. [PubMed: 4041227]
- Erskine MS. Solicitation behavior in the estrous female rat: a review. *Horm. Behav.* 1989; 23:473–502. [PubMed: 2691387]
- Everett JW. Central neural control of reproductive functions of the adenohypophysis. *Physiol. Rev.* 1964; 44:373–431. [PubMed: 14175665]

- Feder HH, Marrone BL. Progesterone: its role in the central nervous system as a facilitator and inhibitor of sexual behavior and gonadotropin release. *Ann. N. Y. Acad. Sci.* 1977; 286:331–354. [PubMed: 31125]
- Feder HH, Ruf KB. Stimulation of progesterone release and estrous behavior by ACTH in ovariectomized rodents. *Endocrinology.* 1969; 84:171–172. [PubMed: 4302292]
- Foreman MM, Moss RL. Effects of subcutaneous injection and intrahypothalamic infusion of releasing hormones upon lordotic response to repetitive coital stimulation. *Horm. Behav.* 1977; 8:219–234. [PubMed: 405307]
- Franck JAE. Short latency induction of lordosis by ether in estrogen-primed female rats. *Physiol. Behav.* 1977; 19:245–247. [PubMed: 564520]
- Goldfoot DA, Goy RW. Abbreviation of behavioral estrus in guinea pigs by coital and vagino-cervical stimulation. *J. Comp. Physiol. Psychol.* 1970; 72:426–434.
- Gorzalka BB, Moe IV. Adrenal role in proceptivity and receptivity induced by two modes of estradiol treatment. *Physiol. Behav.* 1994; 55:29–34. [PubMed: 8140170]
- Gray DS, Gorzalka BB. Adrenal steroid interactions in female sexual behavior: a review. *Psychoneuroendocrinology.* 1980; 5:157–175. [PubMed: 6248915]
- Hardy DF, DeBold J. Effects of coital stimulation upon behavior of the female rat. *J. Comp. Physiol. Psychol.* 1972; 78:400–408. [PubMed: 5067071]
- Hardy DF, Debold JF. Effects of repeated testing on sexual behavior of the female rat. *J. Comp. Physiol. Psychol.* 1973; 85:195–202. [PubMed: 4744482]
- Komisaruk, BR. Inductions of lordosis in ovariectomized rats by stimulation of the vaginal cervix: hormonal and neural interrelationships. In: Sawyer, CH.; Gorski, RA., editors. *Steroid Hormones and Brain Functions.* Berkeley Calif: U. California Press; 1971. p. 127-135.-135.
- Komisaruk BR, Adler NT, Hutchinson J. Genital sensory field: enlargement by estrogen treatment in female rats. *Science.* 1972; 178:1295–1298. [PubMed: 4640068]
- Larsson K, Feder HH, Komisaruk BR. Role of the adrenal glands, repeated matings and monoamines in lordosis behavior of rats. *Pharmacol. Biochem. Behav.* 1974; 2:685–692. [PubMed: 4431830]
- Lodder J, Zeilmaker GH. Role of pelvic nerves in the postcopulatory abbreviation of behavioral estrus in female rats. *J. Comp. Physiol. Psychol.* 1976; 90:925–929. [PubMed: 987072]
- Meerts SH, Clark AS. Artificial vaginocervical stimulation induces a conditioned place preference in female rats. *Horm. Behav.* 2009; 55:128–132. [PubMed: 18848563]
- Meyerson BJ, Lindstrom LH. Sexual motivation in the female rat. *Acta Physiol. Scand. Suppl.* 1973; 389:1–80. [PubMed: 4518829]
- Morin LP. Progesterone: inhibition of rodent sexual behavior. *Physiol. Behav.* 1977; 18:701–715. [PubMed: 331359]
- Peters LC, Kristal MB, Komisaruk BR. Sensory innervation of the external and internal genitalia of the female rat. *Brain Res.* 1987; 408:199–204. [PubMed: 3594208]
- Pfaff DW, Kow LM, Loose MD, Flanagan-Cato LM. Reverse engineering the lordosis behavior circuit. *Horm. Behav.* 2008; 54:347–354. [PubMed: 18539279]
- Pfaus JG, Kleopoulos SP, Mobbs CV, Gibbs RB, Pfaff DW. Sexual stimulation activates *c-fos* within estrogen-concentrating regions of the female rat forebrain. *Brain Res.* 1993; 624:253–267. [PubMed: 8252398]
- Pfaus JG, Smith WJ, Byrne N, Stephens G. Appetitive and consummatory sexual behaviors of female rats in bilevel chambers II. Patterns of estrus termination following vaginocervical stimulation. *Horm. Behav.* 2000; 37:96–107. [PubMed: 10712862]
- Pfeifle JK, Edwards DA. Midbrain lesions eliminate sexual receptivity but spare sexual motivation in female rats. *Physiol. Behav.* 1983; 31:385–389. [PubMed: 6635009]
- Phoenix CH, Copenhaver KH, Brenner RM. Scanning electron microscopy of penile papillae in intact and castrated rats. *Horm. Behav.* 1976; 7:217–227. [PubMed: 955583]
- Powers JB. Hormonal control of sexual receptivity during the estrous cycle of the rat. *Physiol. Behav.* 1970; 5:831–835. [PubMed: 5535480]
- Rajendren G, Dudley CA, Moss RL. Role of the vomeronasal organ in the male-induced enhancement of sexual receptivity in female rats. *Neuroendocrinology.* 1990; 52:368–372. [PubMed: 2124662]

- Rajendren G, Moss RL. The role of the medial nucleus of amygdala in the mating-induced enhancement of lordosis in female rats — the interaction with luteinizing hormone-releasing hormone neuronal system. *Brain Res.* 1993; 617:81–86. [PubMed: 8374748]
- Ramos SM, Debold JF. Protein synthesis in the medial preoptic area is important for the mating-induced decrease in estrus duration in hamsters. *Horm. Behav.* 1999; 35:177–185. [PubMed: 10202125]
- Reading DS, Blaustein JD. The relationship between heat abbreviation and neural progesterin receptors in female rats. *Physiol. Behav.* 1984; 32:973–981. [PubMed: 6541799]
- Rodriguez-Sierra JF, Crowley WR, Komisaruk BR. Vaginal stimulation in rats induces prolonged lordosis responsiveness and sexual receptivity. *J. Comp. Physiol. Psychol.* 1975; 89:79–85. [PubMed: 1159114]
- Roy MM, Goldstein KL, Williams C. Estrus termination following copulation in female guinea pigs. *Horm. Behav.* 1993; 27:397–402. [PubMed: 8225261]
- Sachs BD, Glater GB, O'Hanlon JK. Morphology of the erect glans penis in rats under various gonadal hormone conditions. *Anat. Rec.* 1984; 210:45–52. [PubMed: 6486482]
- Tennent BJ, Smith ER, Davidson JM. The effects of estrogen and progesterone on female rat proceptive behavior. *Horm. Behav.* 1980; 14:65–75. [PubMed: 7399422]
- Tetel MJ, Getzinger MJ, Blaustein JD. Fos expression in the rat brain following vaginal-cervical stimulation by mating and manual probing. *J. Neuroendocrinol.* 1993; 5:397–404. [PubMed: 8401563]
- VanderSchoot P, Vanophemert J, Baumgarten R. Copulatory stimuli in rats induce heat abbreviation through effects on genitalia but not through effects on central nervous mechanisms supporting the steroid hormone-induced sexual responsiveness. *Behav. Brain Res.* 1992; 49:213–223. [PubMed: 1388815]
- Whalen RE. Estrogen–progesterone induction of mating in female rats. *Horm. Behav.* 1974; 5:157–162. [PubMed: 4847184]

Fig. 1.
Diagram depicting the timeline of Experiments 1 and 2.

Fig. 2. (A) Lordosis quotient (LQ), (B) lordosis intensity, (C) ear-wiggling, and (D) rejection behaviors (mean $\pm$ SEM) in masked ($n = 11$) and unmasked ($n = 10$) OVX/ADX females exposed to sexually vigorous males every 30 min.

Fig. 3. (A) Lordosis quotient (LQ), (B) lordosis intensity, (C) ear-wiggling, (D) hops and darts, and (E) rejection behaviors (mean $\pm$ SEM) in masked ($n = 9$) and unmasked ($n = 8$) OVX/ADX females exposed to sexually vigorous males every 30 min.

Fig. 4. Lordosis quotient (LQ) and numbers of rejections (mean $\pm$ SEM) of ovariectomized rats, which received repetitive VCS (VCS+) or scapular stimulation (VCS-) by the experimenter with a plastic probe delivering either 300 g of pressure (top panel) or 150 g of pressure (bottom panel), as described in text.