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Clitoral anesthesia disrupts paced copulation in the female rat

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HIGHLIGHTS

- Clitoral anesthesia decreases the time females spend with males during paced mating.
- Clitoral anesthesia increases the number of approaches towards males.
- Clitoral stimulation is involved in detecting stimuli differences critical to pacing.

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ABSTRACT

Clitoral stimulation produced by sexual contact with a partner or during manual stimulation is associated with pleasure in humans, and produces conditioned place preference in rats. The present experiment investigated the effect of blocking genitosensory stimulation of the clitoris with lidocaine during copulation in female rats on a measure of female sexual motivation: pacing behavior. Sexually naïve, ovariectomized female rats were treated with 10 μg estradiol benzoate 48 h and 500 μg progesterone 4 h prior to a 30-min copulatory trial with a sexually vigorous stimulus male scheduled every 4 days. A total of 10 copulatory sessions were divided into two phases of 5 trails each. In the first phase, females received an injection (0.05 ml) of either 2% lidocaine, saline, or no injection to the clitoral sheath under isoflurane anesthesia immediately prior to the start of a copulatory session, and were then placed on one side of a paced mating chamber and allowed to copulate for 30 min. In the second phase, females previously injected with lidocaine were switched to saline and vice versa, and the no injection group remained the same. Variables measured included overall time spent with the males, number of solicitations, contact–return latencies following male mounts, intromissions, and ejaculations; the frequency of entrances and exits from the male chamber, and frequency of mounts, intromissions, ejaculations. Sexual behavior was examined at session 1, session 5, and session 10. At test 5, females that received LID had a greater number of entrances/exits but spent significantly less time in the presence of the male during the copulatory bout than CNTL animals. These females also displayed a trend for longer contact return latencies s after ejaculations than VEH and CNTL groups. On session 10, females that received LID and subsequently switched to VEH treatment no longer differed from controls in entrance/exit numbers, time spent with males or ejaculation contact return latency. They did however, receive a greater number of intromissions and displayed shorter inter intromission intervals compared to CNTLs. We suggest that clitoral stimulation in the rat serves as both a reward signal and may contribute to the detection of differences in copulatory stimuli that are critical to pacing and potentially, the initiation of pregnancy.

1. Introduction

In natural and laboratory environments female rats control the rate of copulatory stimulation they receive from males by means of approach and avoidance behaviors, collectively known as "pacing" [1–3]. Pacing behavior increases the likelihood that mating stimulation will initiate luteal function thus enhancing the probability of pregnancy when the female is paired with a fertile male or pseudopregnancy if the male in infertile [4–18]. The ability of females to pace or control the initiation and rate of sexual stimulation also leads to a positive reward state that induces both conditioned place and partner preferences [9–13]. Furthermore, it has been suggested that the rewarding aspects of pacing are associated with functional consequences, e.g., reproductive success [14].

In general, pacing rate (the time between solicitations by the female) increases as a function of the specific type of sexual stimulation that she receives. Contact–return latencies (CRLs) are longest following ejaculations, which provide the greatest amount of stimulation from deep thrusting, and shortest following mounts, which provide the least amount of stimulation [15–19]. These data suggest that females differentiate between mounts, intromission, and ejaculations. Intromissions

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and ejaculations both provide vaginocervical stimulation (VCS), an important component of the genitosensory stimulation that females receive during copulation.

Whether supplied during sexual interactions or manually, VCS facilitates lordosis and pacing behaviors, induces analgesia, and facilitates estrus termination [20–26]. Vaginocervical stimulation also induces the release of luteinizing hormone, oxytocin, and prolactin, which are associated with ovulation and pregnancy [27,28]. Stimulation from intromissions and manually applied VCS activates pressure receptors in the vaginal canal, cervix, and uterus, which activate the hypogastric, pelvic, and vagus nerves [29–32]. Manual VCS alone has been shown to induce a reward state strong enough for the development of conditioned place preference, when it is administered at intervals that females prefer [33], which highlights its importance in the reward value of sexual stimulation received by females.

Vaginocervical stimulation is not the only form of tactile stimulation that female rats receive during sexual interaction, however. Stimulation of the flanks, rump, and tail base during male mounts aid in the induction of the lordosis reflex enabling males to gain vaginal intromission and ejaculation. The lordosis posture also allows contact between the lower perineal area of the female and the genitals of the male during male thrusting [34] and this lower perineal contact stimulates the clitoris and, in turn, the pudendal nerve [35].

Studies of the effects of clitoral stimulation in humans have largely focused on its function as a relay of pleasurable sensory information [36–38] and, more recently, on its influence on vaginal muscle function [39]. Shafik et al. [39] recorded vaginal electromygraphic (EMG) activity and pressure changes in response to electrical or mechanical glans clitoris stimulation and demonstrated that stimulation of the glans clitoris significantly increased vaginal EMG and pressure. It is suggested that these measures are an indication of vaginal wall contraction and may affect penile arousal and female sexual stimulation during intercourse [39]. In the rat, clitoral innervation and vasculature during sexual arousal has been studied [40–43] along with its morphology following hormone administration [44–47].

It has been established that stimulation of the clitoris is rewarding to the female rat. Manually applied clitoral stimulation (CLS) that is given in a distributed manner has been shown to induce a positive hedonic state that is strong enough to induce a conditioned place preference compared to when it is given in a continuous manner [48]. The positive hedonic state induced by CLS can also be associated with cues on a particular copulatory partner, such that it induces a solicitational preference for that partner when a female has the choice to copulate with the partner associated with rewarding CLS versus a partner not associated with CLS [49]. The development of this preference is context dependent. In an environment that prevents a female from interacting with a copulatory partner via a mesh wall manual distributed CLS induces a negative reward state that will result in a preference for partners that are not associated with CLS [50].

Evidence also exists indicating that precopulatory CLS influences proceptive behaviors. Manually applied, continuous CLS given prior to a copulatory session with a male partner increased proceptive behavior compared to manually applied distributed CLS but not compared to controls. This finding occurs in sexually naïve ovariectomized female rats that received hormone replacement. No differences in sexual behavior were observed in gonadally-intact females that received manually applied precopulatory CLS, however manually applied, distributed CLS resulted in enhanced fertility only in females that received greater than 9 intromissions [51].

Finally, the effects of CLS are mediated by the level of previous sexual experience that females have. In a recent study, Parada, Jafari, and Pfaus [50] showed that CLS did not induce conditioned place preference in females that received 5 paced copulatory sessions with a male conspecific prior to the conditioning sessions compared to those that received 0 or 1 previous sexual experiences. Sexual experience appears to reduce the reward value of CLS in the sexually naïve female.

The present study examined the role of clitoral stimulation in female sexual behavior in rats through clitoral anesthesia using the local anesthetic agent lidocaine hydrochloride. Females were injected with lidocaine (LID) or saline vehicle (VEH) in the clitoral sheath immediately prior to 5 copulatory sessions with a sexually vigorous male. Female solicitations, pacing, and lordosis behaviors were examined at session 1 and at session 5 between the lidocaine, vehicle and no injection controls (CNTL). A second set of 5 reversal sessions followed, during which females previously in the lidocaine group were given vehicle injections and those previously in the vehicle group were given lidocaine injections. The same behaviors were observed and compared at session 10 to investigate any permanent effects of the anesthetic on sexual behavior and to examine the effect of Lidocaine on females previously injected with vehicle. We hypothesized that blocking rewarding sensory input to the clitoris would result in an increase in solicitations and a possible increase in the time spent with males during the copulatory tests as an attempt to gain more stimulation. This hypothesis is based on observations of female–male mounting (FMM) in female rats paired with castrated non-copulating males, which suggests that FMM is a super-solicitational behavior [52]. We also hypothesized that any effect of the anesthetic on sexual behavior in the first 5 trials would dissipate by the 10th session after several vehicle treatments in females reversed from lidocaine to vehicle. Finally, we hypothesized that females initially treated with vehicle and given lidocaine during the reversal sessions would show an increase in solicitations at the end of the reversal phase.

2. Materials and method

2.1. Animals and surgery

Sexually naïve female Long-Evans rats, weighing 200 to 250 g, were obtained from Charles River Canada, Inc., St. Constant, QC. Animals were pair-housed in shoebox cages in a colony room maintained on a reversed 12:12 h light/dark cycle (lights off at 08:00 h) at approximately 21 °C. Food and water were continuously available. Females were ovariectomized bilaterally through lumbar incisions under intraperitoneal (i.p.) injections (1 ml/kg of body weight) of ketamine hydrochloride (50 mg/ml) and Xylazine hydrochloride (4 mg/ml) anesthetic mixed in a ratio of 4:3 respectively. All females were given 1 week of post surgical recovery and maintained for the duration of the experiment on hormone replacement by subcutaneous injections of estradiol benzoate (EB; 10 μg in 0.1 ml of sesame oil) 48 h and progesterone (P; 500 μg in 0.1 ml of sesame oil) 4 h prior to testing.

2.2. Male sex training

Long-Evans males from the same breeder were given 10, 30-min sessions of sexual training with different sexually receptive females prior to testing in order to generate stable baseline rates of sexual responding ($n = 20$ per group). Males were considered viable copulators if they mounted females within 15 s of the presentation of the female.

All animal procedures conformed to the guidelines of the Canadian Council on Animal Care, and were approved by the Concordia University Animal Research Ethics Committee.

2.3. Injections and testing procedure

Thirty-six females ($n = 12$ per group) were assigned to one of three treatment groups (group names represent treatment in phase 1 followed by treatment in phase 2): Lidocaine–vehicle (LID–VEH), vehicle–lidocaine (VEH–LID), and controls (CNTL). All females experienced a total of 10, 30-min copulatory testing sessions with a random male every 4 days. With the exception of the CNTL group, females received an injection into the clitoral sheath prior to each testing session. The testing sessions were divided into two phases. Phase 1 consisted of 5 testing sessions, which examined the effect of lidocaine or vehicle