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Characterization of copulatory behavior in female mice: Evidence for paced mating

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ABSTRACT

In this study we characterized female mouse sexual behavior using a pacing paradigm similar to that used to evaluate sexual behavior in female rats. A pacing chamber was designed for use with mice and we compared the sexual behavior of female mice that were tested in both pacing and nonpacing paradigms and under different hormone conditions. We found that, like rats, female mice do pace their copulatory behavior by altering the temporal sequence of copulatory events. Female mice take longer to return to the male after an ejaculation, compared to either a mount or intromission. However, it is still unclear if female-paced mating serves the same functions as it does in female rats. More work is needed to confirm that paced mating induces hormonal changes needed for pregnancy as is the case in rats.

1. Introduction

Sexual behavior has been carefully characterized in both male and female rats. However, mouse reproductive behavior has only been fully described for males [1,2]. Female rat sexual behavior has been well studied, but it is unclear that the rat model of sexual behavior is applicable to mice [3,4]. Mouse mating strategies differ from those of the rat. Male mice defend a territory and mate with females in it, excluding other males [5]. But in rats, a single female is likely to mate concurrently with several males [6–8]. Male rats and mice also exhibit different copulation patterns. Copulation in the rat is much shorter, consisting of a series of relatively few intromissions, leading to ejaculation [9]. Whereas copulation in mice consists of numerous intromissions with sustained intravaginal thrusting [9]. The mouse ejaculatory reflex is also different from that of the rat. In male mice, ejaculation leads to a shudder while maintaining intromission. The male then clutches the female with all four limbs and usually falls to his side, frequently carrying the female with him; he remains like this, fully intromitted for 13–25 s [9,10]. During mating, female mice may receive more vaginal/cervical stimulation than female rats, and as a result they are likely to differ from female rats in situations in which they control the pace of copulation in response to coital stimulation.

The goal of the present study was to characterize normative female mouse sexual behavior under several hormonal conditions and testing paradigms. Particularly, we were interested in determining the patterns of behavior female mice exhibit when they are able to control the pace of copulation. We also investigated how the testing paradigm interacted with the hormonal conditions that represent early proestrus (only estradiol) or late proestrus (estradiol plus progesterone). In rats mating can start just before the rise in progesterone, but females are most receptive when progesterone is high [4].

2. Materials and methods

2.1. Animals

Female Swiss Webster mice (Charles River Laboratories, Raleigh, North Carolina) were group housed 4–5 per cage, and male C57BL/6 mice (Charles River Laboratories, Raleigh, North Carolina) were singly housed in clear Plexiglas cages (27.5×17×12 cm). Animals were provided with food (Harlan Teklad 22/5 Rodent Diet 8640) and water *ad libitum*, and maintained on a reverse light dark cycle with lights off from 1100 to 1900 h. All experiments were performed in compliance with the Michigan State University All University Committee on Animal Use and Care, in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize any discomfort experienced by the animals.

2.2. Hormone manipulations

All female mice were ovariectomized via bilateral incisions under ketamine/xylazine anesthesia (1 ml/kg/bw of cocktail 44 mg ketamine/10 mg xylazine/ml). The females were divided into three

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hormone treatment groups. Injections were given at 0900 h. Treatment 1 (EB+P), Estradiol Benzoate (EB, Sigma) .5 ug/.03 ml in oil vehicle was given subcutaneously on days 1, 2, and 3. On the fourth day Progesterone (P, Sigma) was given at .5 mg/.03 ml in sesame oil vehicle, 4 h prior to behavior testing. Treatment 2 (EB only), EB was given to females on days 1, 2 and 3 at the same concentration as treatment 1. Sesame oil was given on day 4 instead of P. Treatment 3 (OIL, Sigma), consisted of only sesame oil vehicle injections of .03 ml subcutaneously on days 1, 2, 3 and 4. On the fourth day, all animals were tested for sexual behavior.

2.3. Behavior testing

After at least a week of recovery following ovariectomy, all animals were allowed to gain sexual experience prior to testing by pairing males and females overnight in the males' homecage. For these pairings, all females were given hormone treatment 1, consisting of EB +P injections to induce sexual receptivity. After sexual experience the females were then randomly assigned to one of the three hormone treatments as described above and tested in both a nonpacing and pacing test, in a counter balanced order. For all tests, females were paired with a male that previously showed reliable sexual behavior and were tested with the same male in both pacing and nonpacing tests. Behavior tests were conducted under dim red illumination at 1300, 2 h after lights out. Males were allowed to acclimate to the testing chambers for 5 min before introducing the female. All behavior tests were videotaped and analyzed with an event recording program (Observer version 2.0). For both pacing and nonpacing conditions, if no intromissions were observed in the initial 20 min the test was ended. Otherwise tests continued until an ejaculation was received in nonpacing tests, or after the female returned to the male following an ejaculation in pacing tests. Only females receiving ejaculations in either a pacing or nonpacing test were included in the statistical analyses.

2.3.1. Female-Paced tests

Pacing chambers used for rats utilize the large sex difference in body size to allow females the opportunity to escape from the male. A large Plexiglas chamber is divided into a “male” chamber and “escape” chamber by a barrier with small holes that males are not able to fit through. Female rats can pass easily through the holes in the divider separating the two chambers, giving them the opportunity to escape the male rat. Male and female mice are approximately the same size, and because rat pacing chambers utilize the size difference between sexes to separate the two we could not use them. Therefore, we designed new chambers for these experiments. The testing chamber consisted of a Plexiglas arena (60 cm×45 cm×45 cm) with a 10 cm tall Plexiglas barrier demarking the male side vs. the female side. The female could easily jump over this barrier to escape the male. The barrier was placed so that the female side was 20×45×45 cm vs. the male side 40×45×45 cm. The males were tethered to the male side of the cage by placing a plastic collar around their neck, and attaching them to a ring affixed above the chamber that swiveled, allowing them access to all areas on the male side, but preventing them from crossing the barrier over to the female side. The females had free access to all areas of the testing chamber, including an area where they could escape and avoid the male.

The frequency of mounts, intromissions, and ejaculations were recorded, as were the latency to the first occurrence of each of these behaviors. Intromissions were further classified as male or female terminated. If the female pushed the male off it was deemed female terminated. If the male dismounted first, then it was deemed male terminated. The inter-intromission interval, or time between intromissions and the duration of each intromission were measured. We also measured how many times the female escaped to the female chamber (percent exits), and also the time for the female to return after each escape (return latency) after a mount, intromission and ejaculation. Lordosis was not reliably shown by the females, and males could intromit without the female displaying lordosis. Our females had limited sexual experience, and were tested only two times, which appears not enough sexual experience to show reliable lordosis in mice. Female mice typically show very low lordosis quotients, around 20% with only 2–3 sexual experiences [11,12]. Instead of lordosis, rejections or acceptances of the male were used as an index of female receptivity. Rejections consisted of the biting and fighting by the female when the male came into contact with her, and were often accompanied by audible vocalizations. Acceptances resulted in mounts or mounts with intromissions.

2.3.2. Nonpacing tests

The testing chamber consisted of the same arena as in the pacing test, but without the barrier. Males remained tethered, but were

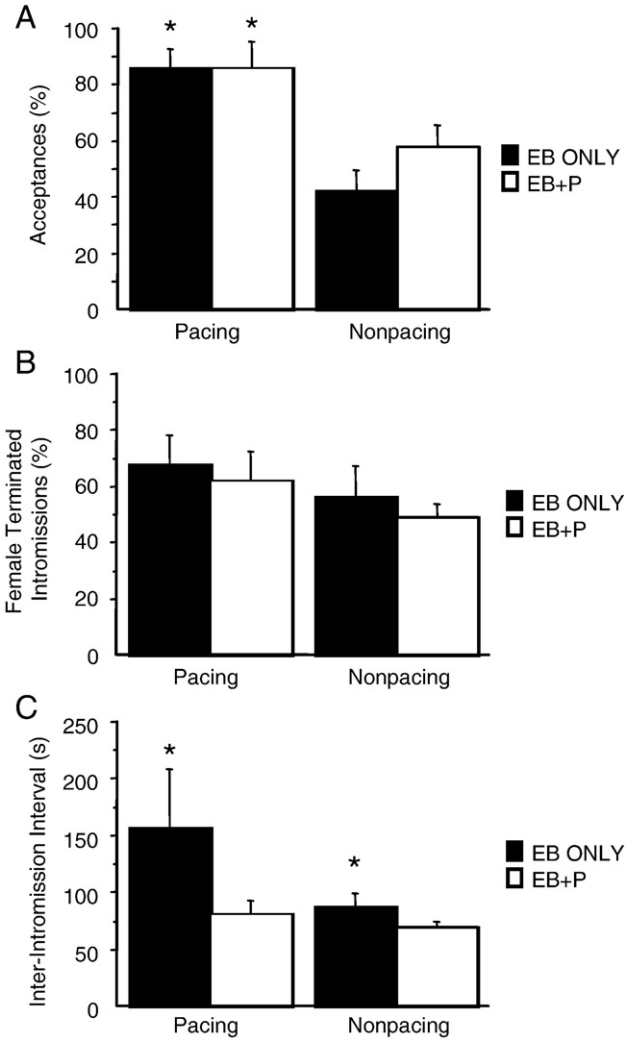

Fig. 1. A) The percentage of male approaches that resulted in acceptances (mounts or mounts with intromissions) by the female, were significantly higher in the pacing test compared to the nonpacing test ($F(1,21)=16.589$, $p=.0005$). There was no main effect of hormone treatment ($F(1,21)=.766$, $p=.39$) or significant interaction ($F(1,21)=.794$, $p=.38$). *Significantly different from nonpacing. B) The percent of intromissions terminated by the females did not differ between testing paradigms ($F(1,21)=1.551$, $p=.2$) or hormonal treatments ($F(1,21)=.446$, $p=.5$) and there was no significant interaction ($F(1,21)=.009$, $p=.9$). C) There was a main effect of testing paradigm ($F(1,21)=4.4$, $p=.048$), and main effect of hormone ($F(1,21)=6.1$, $p=.02$) but no interaction ($F(1,21)=2.28$, $p=.146$) on the inter-intromission interval (III). Post-hoc tests failed to detect a significant effect of testing paradigm, but found that the III was significantly longer in EB only females ($p=.05$) in both testing paradigms. *Significantly longer than EB+P treated females within testing paradigm.