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Cohabitation between male rats after ejaculation: Effects on conditioned partner preference ☆

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HIGHLIGHTS

- Males prefer females that bear cues associated with the postejaculatory interval.
- Exposure to a partner during the PEI facilitates conditioned partner preference.
- We exposed males to other males during the PEI to induce same-sex preference.
- Males failed to form same-sex preference, but developed conditioned hostility.

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ABSTRACT

Male rats display a conditioned ejaculatory preference for females that bear olfactory cues associated with ejaculation + the postejaculatory interval (PEI), or with the PEI alone. This indicates that exposure to a partner during the PEI is necessary and sufficient 'for the development of conditioned sexual partner preference. In the present study we examined the effect of cohabitation between two males during the PEI on the possible development of same-sex partner preference. Males first copulated with an ovariectomized, E + P primed female to one ejaculation and were immediately removed from the female's chamber and placed in another chamber with a conspecific male scented with almond odor as a conditioned stimulus (CS+). Cohabitation lasted for 1 h and started immediately after ejaculation in the PEI group and 7 h later in the control group. Conditioning occurred daily for a total of ten trials with different females, but cohabitation during the PEI occurred always with the same stimulus male partner. On trial 11, males were tested for social partner preference with two stimulus male partners. One was the familiar scented male and the other an unfamiliar unscented male. Results indicated that males did not develop any social or sexual preference for the male associated with the PEI. In fact, rats from the PEI group interacted significantly less with the scented male as compared to the unscented male, and displayed more agonistic behaviors towards the scented male than towards the unscented male. These data show that conditioned same-sex preference does not develop as a result of cohabitation during the PEI. We discuss the implications for conditioned hostility in intrasexual competition.

1. Introduction

Partner preferences commonly occur towards individuals of the opposite sex and are observed with selective contacts, more time spent together and directed courtship behavior and copulation. Interestingly, in many species there are individuals who display some of these behaviors towards members of the same sex, suggesting that the underlying biological mechanisms that mediate partner preference do not necessarily correspond exclusively to those that underlie reproductive purposes. In fact, several studies on laboratory rodents indicate that the development and expression of a preference depend not only

☆ The experimental protocols in this study were approved by a committee of the Graduate Program in Neuroethology, Universidad Veracruzana, Mexico, following the Official Mexican Standard NOM-062-ZOO-1999 (Technical Specifications for the Production, Care and Use of Laboratory Animals).

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on genetic and hormonal mechanisms during the perinatal period, but also on several episodes of learning that occur after birth (for review see [1–3]).

With regard to the effects of learning, recent evidence obtained from our laboratory indicates that rats can display same-sex social partner preference after three conditioning trials in which they received an injection of the dopamine (DA) D2-type receptor agonist (quinpirole) and were allowed to cohabit with another male (scented with almond extract as a conditioned stimulus CS+ to facilitate recognition) [1,2]. Males that experienced same-sex cohabitation under the influence of quinpirole (but not saline) weakened their innate sexual preference for a sexually receptive female in future encounters, and strengthened their preference to be in social and sexual contact with the familiar male that bore the CS+. The preference was observed several days after the last treatment with quinpirole, and included more time spent together (>70% of the test), more body contacts, more female-like proceptive behaviors, more mount attempts, and more non-contact erections evoked by the presence of the conditioned preferred male. It is interesting that the pharmacological enhancement of D2-type activity facilitated the development of such conditioned same-sex partner preference, although to date, it is unknown if this occurs naturally.

More than one decade ago it was also shown that odors associated with a natural rewarding stimulus such as ejaculation can facilitate motivation and preference for a partner [3]. Kippin and colleagues trained one group of males (the paired group) to associate an almond or lemon odor painted on the back of a female's neck and anogenital region with copulation to ejaculation. Another group (the unpaired group) received copulatory trials with unscented females. Both odors have been shown to be neutral before conditioning (do not direct partner preference *per se*, unless paired in contingency with a reinforcer). On a final test in a laboratory open field the males had access to two sexually receptive females, one scented with the odor (now conditioned) and the other unscented. Males in the paired group displayed a conditioned partner preference in which the scented females were chosen to receive the males' first ejaculation. Subsequent studies by the authors revealed that the learning of this conditioned ejaculatory preference took place during the postejaculatory interval (PEI) [4]. The PEI refers to the period that occurs after ejaculation, before the next mount or intromission, during which the male enters an inhibitory phase (or refractory period) in which he does not show any appetitive sexual behavior. Thus, during ejaculation and the subsequent PEI males experience a complex set of neurochemical and neuroendocrine events that are of sufficient magnitude to consolidate reward-related learning about the partner they are with, but that inhibit further sexual behavior [5]. This state appears to involve the release of opioids, endocannabinoids, and serotonin, but also the sensitization of mesolimbic dopamine that becomes conditionally released in the presence of the CS [6]. Likewise, in the our previous studies [1,2], we discussed that male rats that were allowed to cohabit with another male under the effects of quinpirole did develop a same-sex social preference by the association of the conditioned odor bore by the male partner and the enhanced D2-type receptor activity. Thus, based on the fact that laboratory rodents can learn to display same-sex preference, and that the PEI is a natural stimulus that appears to be necessary and sufficient to support conditioning for a partner, we tested whether cohabitation between male rats during the PEI would be sufficient to condition same-sex social and/or sexual partner preference. The existence of a preference of this type would indicate that same-sex preference additionally develops as a result of learning by association of a partner with natural rewards like the PEI.

2. Materials and methods

2.1. Animals

Forty Wistar male rats were used (weight 250–300 g). They were locally bred in our colony room. For the purpose of our study they were randomly categorized as either stimulus (they functioned exclusively as partners during cohabitation) or experimental (to be conditioned). Stimulus rats were sexually experienced (had at least 10 previous sexual encounters to ejaculation with sexually receptive females) and were housed in groups of 5. Stimulus males did not copulate during the conditioning trials to prevent them from experiencing PEI or neurochemical and neuroendocrine events. Experimental rats were sexually naive at the start of the experiment and were housed individually (except during conditioning). In the previous study we showed the importance of sexual experience in the stimulus males [1,2]. All the animals were kept in Plexiglas cages with a thin layer of aspen chip (Rismart), and maintained in at room temperature on a reverse 12:12 h light/dark cycle (lights off at 08:00 h), at the Centro de Investigaciones Cerebrales, Universidad Veracruzana, Xalapa, Veracruz, Mexico. Water and rodent feed (Rismart) were provided *ad libitum*.

Twenty Wistar females were also used as sexual partners during the conditioning trials of the experimental males. First they were ovariectomized (OVX) and then primed with hormones to induce sexual receptivity during the conditioning trials. Before ovariectomy they were first anesthetized with ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (4 mg/ml), mixed at a ratio of 4:3, respectively, and injected intraperitoneally in a volume of 1 ml/kg of body weight. Anesthetized females were then OVX bilaterally *via* a lumbar incision. Post-surgical treatment included five days of subcutaneous injections of flunixin meglumine (2.5 mg/kg) for analgesia, and enrofloxacin (5 mg/kg) every 24 h to prevent post-surgical bacterial infections. All females were given a week of post-surgical recovery. Sexual receptivity was induced by subcutaneous administration of estradiol (10 μg) 48 h prior and progesterone (500 μg) 4 h prior to each test trial. Females were housed under the same conditions as males (groups of 5). Females were randomly selected to mate with the experimental males during the conditioning sessions, and were prevented from copulating with the same male more than once.

2.2. Partner conditioning

Experimental males were allowed to copulate to one ejaculation with sexually receptive females in a cylindrical copulatory chamber. One group of experimental males was referred to as "PEI group" because cohabitation with a scented stimulus male started immediately after ejaculation during the postejaculatory interval. A second group was referred to as "control" because cohabitation with a scented stimulus male started 7 h after ejaculation (sufficient time to avoid contingency between the postejaculatory period and exposure to the odor). Both males cohabited in a clean and novel medium size Plexiglas cage (20 cm × 30 cm × 45 cm) with aspen chip as bedding, during 1 h. The stimulus male was scented with 0.5 ml of almond extract (Deiman®, Mexico), applied on the back and neck. This was repeated every day for a total of ten trials. At the eleventh day they were tested for a partner preference between the scented male partner and a novel unscented male.

2.3. Partner preference test

Same-sex social/sexual preference test was carried out one day after the final conditioning trial. The preference test lasted for 20 min and occurred in a three-compartment chamber (20 cm × 30 cm × 45 cm) that had a thin layer of aspen chip. In one goal compartment there was the stimulus familiar male (almond scented) and in the other there was the novel stimulus male (unscented). Both wore rodent jackets connected to an elastic tape of 20 cm in length, which prevented them from roaming around. Each experimental male was placed in the start compartment that was connected to the two goal compartments by a T-shaped transparent tunnel of 20 cm in length. Each experimental male was able to move freely and the stimulus males were allowed to move only in their own compartment, but not beyond.