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Heterosexual experience prevents the development of conditioned same-sex partner preference in male rats☆

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ABSTRACT

Sexual partner preferences can be strengthened, weakened or even drastically modified via Pavlovian conditioning. For example, conditioned same-sex partner preference develops in sexually-naïve male rats that undergo same-sex cohabitation under the effects of quinpirole (QNP, D2 agonist). Here, we assessed the effect of prior heterosexual experience on the probability to develop a conditioned same-sex preference. Naïve or Sexually-experienced males received either Saline or QNP and cohabited during 24 h with a male partner that bore almond scent on the back as conditioned stimulus. This was repeated every 4 days for a total of three trials and resulted in four groups (Saline-naïve, Saline-experienced, QNP-naïve, QNP-experienced). Social and sexual preference were assessed four days after the last conditioning trial in a drug-free test in which experimental males chose between the scented familiar male and a novel sexually receptive female. Results showed that Saline-naïve, Saline-experienced and QNP-experienced displayed a clear preference for the female (opposite-sex). By contrast, only QNP-naïve males displayed a same-sex preference. Accordingly, QNP-experienced males were not affected by the conditioning process and continued to prefer females. We discuss the effects of copulation and D2 agonists on the facilitation and/or disruption of conditioned partner preferences

1. Introduction

Sexual partner preferences can be strengthened, weakened or even drastically modified (Coria-Avila et al., 2014; Coria-Avila, 2012) via Pavlovian conditioning (Pavlov, 1927). Strengthening occurs, for example, when a neutral cue gains incentive value after being associated in contingency and contiguity with an unconditioned stimulus (UCS) that produces a rewarding unconditioned response (UCR). After some repetitions, the neutral cue functions as a predictor of the UCS and becomes a conditioned stimulus (CS) capable of inducing a conditioned response (CR). Accordingly, partner-related cues (e.g. odors, colors, etc.) experienced in the presence of reward (i.e. sex) can evoke a representation of that reward, and thereby become desired features that identify the partner as the preferred one over other potential partners (Pfaus et al., 2012, 2001).

Copulation is a typical UCS that triggers many neuroendocrine mechanisms, which in turn may facilitate the development of conditioned partner preferences in animals (Coria-Avila et al., 2006; Coria-Avila et al., 2005; Kippin and Pfaus, 2001; Kippin et al., 2001; Ismail et al., 2009; Gingrich et al., 2000). For instance, copulation increases the dynamics of neurotransmitters such as dopamine (Pfaus et al., 1990, 1995), opioids (Agmo and Berenfeld, 1990; Paredes and Vazquez, 1999; Paredes and Martinez, 2001; van Furth et al., 1995), oxytocin and vasopressin (Bales et al., 2007; Bielsky and Young, 2004; Carmichael et al., 1987; Carter et al., 1992; Cushing and Carter, 2000; Young and Wang, 2004). Thus, changes in these neurotransmitters may increase or decrease the probability for the development or maintenance of a partner preference (Coria-Avila et al., 2016; Triana-Del Rio et al., 2015). For example, monogamous voles can develop a heterosexual partner preference (pair bond) if they are allowed to cohabit with an individual of

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the opposite sex under the effects of a systemic injection of non-selective dopaminergic drugs (i.e. apomorphine) or selective D2 agonists (but not after D1) (Aragona et al., 2003, 2006; Wang and Aragona, 2004; Wang et al., 1999). Similarly, some experiments in male rats have demonstrated that cohabitation under the effects of a D2-type agonist can be sufficiently powerful to strengthen a conditioned same-sex partner preference (a learned socio-sexual preference for another male) and to weaken the innate preference for a sexually-receptive female. For example, in our laboratory we showed that sexually naïve male rats can learn to prefer another male after a short conditioning process in which they cohabit during 24 h under the effects of quinpirole (QNP, a D2-type receptor agonist). In those studies the experimental male was sexually-naïve and received QNP, whereas the stimulus male was sexually expert and bore an artificial odor that functioned as CS. The partner preference of the experimental male was assessed in a QNP-free final test, four days after a third conditioning trial and before a sexually-receptive female and the familiar almond-scented male as potential partners at the same time. Conditioned males displayed a weak socio-sexual interest towards the female and a strong interest for the male as observed with more visits, more time spent near him or in close body contact, more mounts or mount attempts, more female-like sexual solicitations directed to him, more olfactory investigations and more non-contact erections evoked by his presence (Cibrian-Llanderal et al., 2012; Triana-Del Rio et al., 2011, 2015). Thus, the QNP-induced activation of D2-type receptors during cohabitation can be considered as the UCS that would normally occur during heterosexual copulation (Pfaus et al., 1990; Gingrich et al., 2000). Therefore, cohabitation with another male under the influence of a D2 agonist facilitates socio/sexual motivation (without the need of copulation) and the association with specific partner cues that gain incentive value and crystalize learning after some repetitions.

The process behind the development of these conditioned same-sex preferences appears to be an important mechanism to explore and to study. For instance, the results in rats indicate that sexual partner preferences are not permanently fixed for life, nor are dependent on innate mechanisms only, such as those widely discussed around the brain organizational hypothesis (Phoenix et al., 1959; Gulia and Mallick, 2010; LeVay, 1991; Roselli et al., 2011; Savic et al., 2005; Swaab et al., 1995; Weinrich, 1982; Paredes and Baum, 1995), but that are likely flexible and can be shaped via learning that occurs under enhanced activity of D2 and oxytocin receptors (Triana-Del Rio et al., 2015). Yet, many questions remained unanswered regarding how or when sexual preferences are sensitive to learning. For example, copulation also upregulates D1-type receptors in rats (McHenry et al., 2012), and decreases the number of D2-positive cells that express Fos (a marker of neural activity) (Nutsch et al., 2016). Accordingly, copulation has the potential to either facilitate or inhibit the development of partner preferences. Thus, in the present study we allowed male rats to gain heterosexual experience by copulating during several trials with a female. Then we assessed the probability of those males to develop a conditioned same-sex partner preference during cohabitation with another male under the effects of the D2 agonist QNP. We hypothesized that heterosexual experience would prevent the development of conditioned same-sex socio/sexual preference.

2. Material and methods

2.1. Subjects

Thirty five Wistar (W) male rats were used as experimental subjects to be conditioned and twenty males and twenty females were used as stimulus animals. All of them were purchased from a certified animal supplier (Circulo ADN®) and had similar body weights at the start of the study (250–300 g). Stimulus rats were always housed by sex in groups of five in plexiglas cages with a thin layer of woodchip, whereas experimental rats were housed individually during one week before the start of the study. All the rats were maintained at room temperature on a reverse 12:12 h light/dark cycle (lights off at 08:00 h), at the Center for Brain Research (Centro de Investigaciones Cerebrales) Universidad Veracruzana, Mexico. Water and rodent feed (Purina®) were provided ad libitum.

2.2. Sexual training and surgery in females

Twenty experimental males were sexually-naïve and other fifteen experimental males were sexually-experienced. The latter received 10 trials of sexual training with ovariectomized (OVX) hormone-primed females prior to the start of the conditioning. Sexual training ocurred every four days, in transparent cilindric chambers (50 cm height × 60 cm diameter) with a thin layer of woodchip as bedding. Each male to become sexually-experienced was placed alone in the chamber during 5 min prior to the introduction of a sexually-receptive female. The trial lasted until the first ejaculation was observed, or after 90 min otherwise. For ovariectomy, females were anesthetized with a mixture of ketamine hydrochloride (100 mg/ml) and xylazine hydrochloride (10 mg/ml), injected intraperitoneally (i.p.) in a volume of 1 ml/kg of body weight. Anesthetized females were then OVX bilaterally via a lumbar incision. Post-surgical treatment included rehydration with 0.9% saline (10 ml/kg subcutaneously s.c.) and three days of flunixin meglumine (2.5 mg/kg s.c.) for analgesia, and five days of enrofloxacine (5 mg/kg s.c.) every 24 h to prevent post-surgical bacterial infections. All females were given at least one week of postsurgical recovery before they were used in the experiment. Females were primed with injections of estradiol benzoate (10 μg/s.c.) 48 h and progesterone (500 μg/s.c.) 4 h before every test so that they expressed full sexual receptivity and proceptivity.

2.3. Drugs and groups

Eighteen males were treated with the dopamine D2-type receptor agonist quinpirole dihydrochloride (QNP) (Sigma Mexico). It was disolved in 0.9% physiological saline and was injected i.p. in a dose of 1.25 mg/kg in a volume of 1 ml/kg, 1 min before every conditioning trial. Seventeen rats served as controls and were injected with 1 ml/kg of injectable saline (0.9%) 1 min before conditioning. About half of them were sexually-naïve and the other half were sexually-experienced. This resulted in four groups: Saline-naïve (n = 10), Saline-experienced (n = 7), QNP-naïve (n = 10), QNP-experienced (n = 8).

2.4. Partner conditioning

Four days after the last trial of sexual experience (or the equivalent time in naïve animals) the males started the conditioning process to induce same-sex preference. Every conditioning trial lasted 24 h (beginning at 12:00 h and finishing at 12:00 h of the following day), and occurred every 4 days, for a total of three trials. During conditioning, experimental males received either QNP or Saline. Cohabitation occurred in a plexiglas cage (20 cm × 30 cm × 45 cm) with a sexually-experienced stimulus male rat scented with 0.5 ml of almond extract (Deiman® Mexico), applied on the back and neck. Almond extract functioned as a CS to facilitate olfactory recognition during the final partner preference test. After 24 h of cohabitation, the experimental males were single-caged, and remained undisturbed until the next conditioning trial