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Olfactory conditioned same-sex partner preference in female rats: Role of ovarian hormones☆

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ABSTRACT

The dopamine D2-type receptor agonist quinpirole (QNP) facilitates the development of conditioned same-sex partner preference in males during cohabitation, but not in ovariectomized (OVX) females, primed with estradiol benzoate (EB) and progesterone (P). Herein we tested the effects of QNP on OVX, EB-only primed females. Females received a systemic injection (every four days) of either saline (Saline-conditioned) or QNP (QNP-conditioned) and then cohabited for 24 h with lemon-scented stimulus females (CS +), during three trials. In test 1 (female-female) preference was QNP-free, and females chose between the CS + female and a novel female. In test 2 (male-female) they chose between the CS + female and a sexually experienced male. In test 1 Saline-conditioned females displayed more hops & darts towards the novel female, but QNP-conditioned females displayed more sexual solicitations towards the CS + female. In test 2 Saline-conditioned females displayed a clear preference for the male, whereas QNP-conditioned females displayed what we considered a bisexual preference. We discuss the effect of dopamine and ovarian hormones on the development of olfactory conditioned same-sex preference in females.

1. Introduction

A very recent and comprehensive review on sexual orientation concluded that: "*No specific theory of what causes people to be attracted to men, to women, or to both has received enough support to win the backing of all reasonable scientists, most of whom remain open-minded to a large extent*" (Bailey et al., 2016). Indeed, different approaches on the causes of same-sex orientation have been focused on either Nature or Nurture terms. The Nature mechanisms, also referred to as biological, inherited, innate, natural or essential, are mainly supported by empirical data on the genetic, neuroendocrine and neuroanatomical differences between homosexual and heterosexual individuals (Bailey et al., 1993; Coria-Avila et al., 2014a; Gulia and Mallick, 2010; LeVay, 1991; Ponseti et al., 2009; Roselli et al., 2011; Safron et al., 2007; Savic et al., 2005; Swaab et al., 1995; Weinrich, 1982), although such evidence is not totally precise, specifically, because the complete brain engram of sexual preferences is still unknown and most of those differences in genes, hormones or brain areas are mainly correlates that do not represent a perfect causation of a given sexual preference. On the other hand, the Nurture mechanisms are sometimes referred to as chosen, environmental, not hormonal, learned, acquired, socialized, unnatural or socially constructed (Bailey et al., 2016). Nurture mechanisms are often based on less robust evidence, varying from psychoanalytical interpretations about early boyhood traumas (Nicolosi, 1997; Nicolosi and Byrd, 2002), or as a consequence of molestation by older same-sex individuals (Fejes, 2008), or the result of erotic attraction towards dissimilar peers in childhood, the so-called *Exotic Becomes Erotic* theory (Bem, 1996). Interestingly, there is plenty of empirical evidence in many species about the effects of learning on the expression of partner preferences via Pavlovian conditioning. This type of learning process occurs when an association is formed between a conditioned stimulus (CS) and an unconditioned stimulus (UCS) that causes an unconditioned response (UCR), which in turn modifies the perception of sexual incentive on the potential mates based on the presence of the CS (Pfaus et al., 2001). For example, female rats can display a conditioned partner preference for males that bear a conditioned odor (i.e. almond extract) previously paired with the sexual reward induced by paced copulation (Coria-Avila et al., 2005). In that case, the almond odor works as CS,

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whereas paced copulation and its consequences (i.e. sexual reward) function as UCS and UCR, respectively.

The contingency between CS and UCS that results in conditioned partner preference is modulated to some extent by dopamine (DA). For example, female rats that receive a systemic low dose of the DA antagonist flupenthixol before paced copulation, fail to develop any conditioned preference for a male that bears the conditioned odor (Coria-Avila et al., 2008). In that case, it was argued that flupenthixol blocked incentive learning about reward-related cues. By contrast, DA agonists can facilitate the development of conditioned partner preferences because they can enhance the sexual incentive on the partner, even in the absence of explicit sexual reward. For example, low doses of apomorphine facilitate pair bonding in monogamous voles (*M. ochrogaster*) that cohabit in absence of copulation (Aragona et al., 2003; Wang et al., 1999). In addition, brain studies indicate that DA D2-type receptor activity in the rostral shell of the nucleus accumbens (NAcc) is critical for pair bonding since blockade of NAcc DA with the D2-type receptor antagonist eticlopride prevents the formation of pair bonding after mating or after 24 h of cohabitation (Aragona et al., 2006; Gingrich et al., 2000), and female voles infused with the D2-type receptor agonist quinpirole (QNP), develop a pair bond towards a familiar male even during short periods of cohabitation, or without mating (Gingrich et al., 2000).

In a series of experiments we have demonstrated that QNP not only facilitates the development of heterosexual partner preference, but also facilitates the development of same-sex preference between male rats if the correct contingency occurs. To be precise, one male injected with QNP and then allowed to cohabit with another untreated male (Cibrian-Llanderal et al., 2012b; Diaz-Estrada et al., 2015; Triana-Del Rio et al., 2011; Triana-Del Rio et al., 2015). In this type of studies, experimental male rats receive systemic QNP 1 min before they are placed to cohabit with another male that bears almond scent as CS. As expected, they do not copulate during cohabitation. However, treatment with QNP is believed to mimick the dopamine-dependent UCR during motivation in a typical heterosexual mating encounter (Gingrich et al., 2000; Pfaus et al., 1990; Pfaus and Phillips, 1991), which results in higher attention and more incentive towards the same-sex partner. After 24 h of cohabitation both experimental and stimulus males are returned to their single home cages, and the process is repeated again four days later, for a total of three trials. The final preference test occurs four days after the last conditioning trial, namely when the injected QNP has been completely metabolized and has no more acute effects. Experimental males get to choose their preferred partner in a t-shaped chamber that contains in one side the familiar male that bears the olfactory CS, and in the other side a sexually receptive female. Conditioned same-sex preference is observed with a higher frequency of behaviors that represent sexual arousal (i.e. non-contact erections) and social interest (e.g. body contacts, time spent together, visits, olfactory investigations, mount attempts, etc.) directed towards the male, and not towards the receptive female. Accordingly, we have argued that sexual preferences are not completely fixed in adulthood, but can be flexible and therefore possibly rewired depending on how brain dynamics (e.g. dopaminergic activity) can support new learning (Coria-Avila et al., 2016; Coria-Avila et al., 2014b). In this case, facilitated by the effect of enhanced D2-type activity (QNP) during same-sex cohabitation.

As mentioned above, we have successfully induced this type of conditioned same-sex preference in males (Cibrian-Llanderal et al., 2012b; Diaz-Estrada et al., 2015; Triana-Del Rio et al., 2011; Triana-Del Rio et al., 2015), but not in ovariectomized (OVX) females primed with estradiol benzoate (EB) and progesterone (P), regardless of the dose of QNP. In our preceding study, experimental females were OVX and sexually receptive with EB + P during the conditioning trials (cohabiting under the effect of QNP with another female) and were also receptive (EB + P) during the final preference test (QNP-free) in which they chose between two sexually receptive females as potential partners. Contrary to what is observed in males, those females failed to express any conditioned preference (absence of social or proceptive sexual behaviors selectively directed towards the female bearing the olfactory CS) (Triana-Del Rio et al., 2011). Interestingly, evidence indicates that EB + P females express a higher D1:D2 receptor ratio in brain areas like the medial preoptic (mPOA), and thus express a D1-mediated system, whereas females treated exclusively with EB have a lower D1:D2 receptor ratio, and thus express a D2-mediated system (Graham et al., 2015). Accordingly, we believe that the neuroendocrine status induced by EB + P during conditioning facilitates the expression of sexual behaviors, but it also disrupts the QNP-induced effect by reducing D2-type receptor availability, resulting in reduced salience for the same-sex partner during conditioning. Thus, in the present study we explored the possible iteration of EB and QNP in a female rat model of learned same-sex preference. We hypothesized that same-sex partner preference would develop in OVX females that receive EB exclusively during the QNP + cohabitation conditioning trials.

2. Methods

2.1. Subjects

Twenty Wistar (W) males and thirty eight W female rats (250–300 g) were purchased from a certified animal supplier (Rismart, Mexico). For the purpose of our study they were randomly categorized as either stimulus or experimental. Stimulus rats (males and females) served as partners and were housed in groups of 4–5, whereas experimental females were housed individually (except during the conditioning trials when they were allowed to cohabit). All the rats were kept in plexiglas cages with a thin layer of wood shaving, and were maintained 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. Water and rodent chow (Lab Diet, Purina) were provided ad libitum. The present study was carried out in accordance with the Mexican Official Norm NOM-062-ZOO-1999, for use and care of laboratory animals.

2.2. Ovariectomy

All the females in this study were ovariectomized (OVX). They were anesthetized with a mixture of ketamine hydrochloride (75 mg/kg) and xylazine hydrochloride (5 mg/kg), 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 three days of subcutaneous injections of flunixin meglumine (2.5 mg/kg) for analgesia, and enrofloxacine (5 mg/kg) every 24 h to prevent post-surgical bacterial infections. All the females were given a week of post-surgical recovery.

2.3. Drugs and hormone treatment

Half of the experimental female rats formed the QNP-conditioned group ($n = 10$). They were treated 1 min before every conditioning trial with the dopamine D2-type receptor agonist quinpirole dihydrochloride (QNP) (Sigma Mexico, catalogue Q111), dissolved in 0.9% physiological saline and injected intraperitoneally in a dose of 1.25 mg/kg [as in (Cibrian-Llanderal et al., 2012b; Triana-Del Rio et al., 2011; Triana-Del Rio et al., 2015; Wang et al., 1999)] in a volume of 1 ml/kg. Peak plasma concentrations of QNP are detected about 15 min after the injection, and within 24 h the majority of the drug (> 50%) is excreted, although it takes 72 h to be undetected in blood (Whitaker and Lindstrom, 1987). Thus, every conditioning trial had a duration of 24 h based on the pharmacokinetics of QNP. The other half of experimental rats were controls and formed the Saline-conditioned group ($n = 9$). They were injected intraperitoneally with 1 ml/kg of physiological saline 1 min before every conditioning trial. In addition, both groups were primed exclusively with Estradiol Benzoate (EB) given via