Hormones and Behavior 99 (2018) 50–56

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Brain activation associated to olfactory conditioned same-sex partner preference in male rats

Genaro A. Coria-Avila[a,*], Tamara Cibrian-Llanderal[b], Victor X. Díaz-Estrada[c], Luis I. García[a], Rebeca Toledo-Cárdenas[a], James G. Pfaus[d], Jorge Manzo[a]

[a] Centro de Investigaciones Cerebrales, Universidad Veracruzana, Mexico
[b] CONACYT, Instituto de Neuroetología, Universidad Veracruzana, Mexico
[c] Maestría en Neuroetología, Universidad Veracruzana, Mexico
[d] CSBN/Psychology, Concordia University, Montreal, QC, Canada

ARTICLE INFO

Keywords:
Sex
Dopamine
Homosexual
Quinpirole
Accumbens
Fos
Learning

ABSTRACT

Sexual preferences can be strongly modified by Pavlovian learning. For instance, olfactory conditioned same-sex partner preference can occur when a sexually naïve male cohabits with an scented male during repeated periods under the effects of enhanced D2-type activity. Preference is observed days later via social and sexual behaviors. Herein we explored brain activity related to learned same-sex preference (Fos-Immunoreactivity, IR) following exposure to a conditioned odor paired with same-sex preference. During conditioning trials males received either saline or the D2-type receptor agonist quinpirole (QNP) and cohabitated during 24 h with a stimulus male that bore almond scent on the back as conditioned stimulus. This was repeated every 4 days, for a total of three trials. In a drug-free final test we assessed socio/sexual partner preference between the scented male and a receptive female. The results indicated that QNP-conditioned males developed a same-sex preference observed via contact, time spent, olfactory investigations, and non-contact erections. By contrast, saline-conditioned and intact (non-exposed to conditioning) males expressed an unconditioned preference for the female. Four days later the males were exposed to almond scent and their brains were processed for Fos-IR. Results indicated that the QNP-conditioned group expressed more Fos-IR in the nucleus accumbens (AcbSh), medial preoptic area (MPA), piriform cortex (Pir) and ventromedial nucleus of the hypothalamus (VMH) as compared to saline-conditioned. Intact males expressed the lowest Fos-IR in AcbSh and VMH, but the highest in MPA and Pir. We discuss the role of these areas in the learning process of same-sex partner preferences and olfactory discrimination.

1. Introduction

Artificial ornaments can alter the perception of attractiveness on a partner, especially if they represent past reinforcing experiences. An example of this occurs during olfactory conditioned partner preferences. This type of learned preferences for an unfamiliar partner occur when a neutral odor (i.e. almond scent) gains incentive value through repeated associations with an unconditioned stimulus (UCS) that produces an unconditioned response (UCR) (Pavlov, 1927; Pfaus et al., 2001). After few trials, the odor functions as the predictor of the UCS and becomes a conditioned stimulus (CS) that induces a conditioned response (CR). In an olfactory conditioned partner preference the CR is commonly expressed via behaviors indicative of more motivation and preference for the partner that bears the odor (Coria-Avila et al., 2005; Ismail et al., 2010; Kippin et al., 2001). Interestingly, the effects of conditioning can be sufficiently powerful to facilitate the preference for same-sex individuals under certain conditions. For instance, in previous experiments we showed that after three trials of same-sex cohabitation under the effects of enhanced dopaminergic activity (treated with the D2-type receptor agonist quinpirole, QNP), sexually-naïve male rats learned to prefer an almond-scented male (as observed via more body contacts, more visits and by spending more time in close contact) and were more sexually-aroused with him (as observed via non-contact erections), than with a sexually receptive female (Cibrian-Llanderal et al., 2012; Triana-Del Rio et al., 2011; Triana-Del Rio et al., 2015). Such conditioned preference was observed some days after the last conditioning trial and without any QNP on board. Accordingly, we have argued that uncommon conditioned preferences (same-sex oriented) can be developed if they are supported by the appropriate brain chemistry during memory consolidation, in this case by the D2-type agonist QNP.

Under natural circumstances, sexual motivation and partner

* Corresponding author at: Centro de Investigaciones Cerebrales, Universidad Veracruzana, Avenida Luis Castelazo s/n Col. Industrial Ánimas, C. P. 91190, Xalapa, Veracruz, Mexico.
E-mail address: gcoria@uv.mx (G.A. Coria-Avila).

https://doi.org/10.1016/j.yhbeh.2018.02.005
Received 18 December 2017; Received in revised form 3 February 2018; Accepted 6 February 2018

preference occur, to some extent, because the preferred partner displays UCS that are sufficiently relevant to elevate the levels of brain dopamine in the beholder, particularly in areas like the nucleus accumbens (Gingrich et al., 2000; Pfaus et al., 1990) and in the medial preoptic area (MPA) (Hull et al., 1997). Therefore, systemic treatment with the D2-type agonist QNP may be sufficient to simulate part of the UCS that normally occurs during sex in heterosexual couples, which is believed to keep the attention and motivation elevated for the partner (Fiorino et al., 1997). For example, treatment with QNP facilitates the development of pair bonds in monogamous voles without the need of mating (Aragona et al., 2003; Aragona et al., 2006; Gingrich et al., 2000; Wang and Aragona, 2004; Wang et al., 1999). D2-type activity in the nucleus accumbens (specifically in the rostral shell, AcbSh) is needed for pair bonding because blockade with the D2-type antagonist eticlopride disrupts its formation after sex or after a prolonged period of cohabitation (Aragona et al., 2006; Gingrich et al., 2000). As mentioned above, and based on a series of behavioral studies, we have observed that under the influence of QNP a male rat can learn to prefer as partner an almond-scented male after some repeated cohabitations. Then, some days later in a QNP-free test, his partner preference can be assessed before the familiar scented-male and a novel sexually receptive female available at the same time. Very consistently, we have observed that QNP-conditioned males display a socio/sexual preference for the almond-scented male they cohabited with, and ignore the sexually receptive female (Cibrian-Llanderal et al., 2012; Triana-Del Rio et al., 2015). The preference is observed through the display of more body contacts, more visits and by spending more time in close contact with him. By contrast, saline-conditioned males fail to develop a preference for the male and (as expected) express an unconditioned preference for the female. In addition, QNP-conditioned males display more non-contact erections when they are exposed to the almond-scented male behind a transparent perforated divider, and display fewer erections (than those observed with the male) when they are exposed to the sexually-receptive female. Non-contact erections indicate that proximity to the scented male evokes more genital arousal than proximity to a receptive female, which indicates changes in the pattern of sexual arousal and may be interpreted as a modification of sexual preference (Bailey and Hsu, 2017). Accordingly, partner preferences are modified after the conditioning process because some brain areas such as AcbSh and MPA may interpret the conditioned olfactory cue (almond) on a male as more salient, along with other brain areas known to process the incentive value of odors and sexual behavior, like the piriform cortex (Coria-Avila and Pfaus, 2007; Pfaus et al., 2009) and the ventromedial hypothalamus (Robarts and Baum, 2007), respectively. Thus, in the present study we examined Fos immunoreactivity (IR) (Pfaus and Heeb, 1997) as an indicator of brain activity in adult male rats following exposure to the conditioned odor only (almond), previously paired with same-sex partner preference. We hypothesized that exposure to the conditioned odor would enhance the expression of Fos-IR in the brain of QNP-conditioned males, but not in saline-conditioned, nor in intact males.

2. General methods

2.1. Subjects

Fos-IR was assessed in 15 brains of Wistar male rats. Those animals were randomly selected from a larger group of rats previously used in a study that assessed the development of olfactory conditioned same-sex preference (Cibrian-Llanderal et al., 2012). Rats were bred in our colony and had similar body weights (250–300 g) at the start of the study. They were sexually naïve and individually housed in Plexiglas cages with a thin layer of aspen chip. In addition, we used stimulus male and female rats that were sexually experienced and were housed by sex in groups of five. Sexual experience is needed in the stimulus groups to facilitate vigor during the tests. However, experimental males were sexually naïve as in our previous reports. We have shown that sexual inexperience is required to facilitate conditioning of same-sex partner preference (Ramirez-Rodriguez et al., 2017). The colony room was 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, Mexico. Water and rodent feed (Rismart) were provided ad libitum. All the experiments were carried out in accordance with the Mexican Official Norm NOM-062-ZOO-1999 for use and care of laboratory animals.

2.2. Drugs

Rats were organized into three groups: 1) Intact, 2) saline-conditioned, and 3) QNP-conditioned. QNP-conditioned rats received the dopamine D2-type receptor agonist quinpirole dihydrochloride (QNP) (Sigma®; St. Louis, MO) dissolved in 0.9% physiological saline and injected intraperitoneally (i.p.) in a dose of 1.25 mg/kg [as in (Cibrian-Llanderal et al., 2012)] in a volume of 1 ml/kg 1 min before every conditioning trial. Saline-conditioned rats served as controls and were injected i.p. with 1 ml/kg of physiological saline (injectable grade) 1 min before every conditioning trial. Intact rats did not receive injections or conditioning trials.

2.3. Olfactory conditioning of same-sex partner preference

During the conditioning trials rats received their treatment (as explained above in *Drugs*) one minute before being placed in a Plexiglas cage (20 cm × 30 cm × 45 cm) that contained a sexually-experienced stimulus male rat. The stimulus male was scented with 0.5 ml of almond extract (Deiman® Mexico), applied on the back and neck. Almond extract served as a CS to facilitate recognition during the partner preference test. The couple cohabited during 24 h (beginning at 12:00 h and finishing at 12:00 h of the following day) and was always paired together. After cohabitation they were returned to their home cages and the process of cohabitation was repeated again four days later, for a total of three trials [as in (Cibrian-Llanderal et al., 2012)]. Male rats that served as stimulus had at least ten trials of sexual experience with ovariectomized (OVX) hormone-primed females prior to the start of the experiment. Likewise, females that served as stimulus were OVX and primed fully (sexually proceptive/receptive) with subcutaneous (sc) injections of estradiol benzoate (10 μg) 48 h and progesterone (500 μg) 4 h before the final preference test. Female proceptivity was confirmed before each test by observing female proceptive behaviors (hops, darts, ear wiggling) in their group homecage. In addition, receptivity was confirmed when females responded with lordosis upon flank stimulation.

2.4. Partner preference test

The preference test occurred four days after the final conditioning trial and it was drug-free. Accordingly, the display of a same-sex preference was not consequence of acute QNP, but the result of a conditioned preference. For the preference test, experimental rats were placed into a three-compartment chamber that had a thin layer of aspen chip. The start compartment (20 cm × 30 cm × 45 cm) was connected to the two goal compartments by a T-shaped transparent tunnel of 20 cm in length. In one side there was the almond-scented male, and in the other there was an unscented sexually receptive female. The two potential partners were tied by an elastic cord 20 cm long, which allowed them to roam within their chamber only. Experimental males were allowed to interact freely with the two partners for 20 min in a drug-free test and social/sexual behaviors were videoscored and analysed (see Table 1). In addition, we assessed genital (sexual) arousal by measuring non-contact erections during 20 min upon exposure to the scented male or the receptive female in two separate tests one day apart. The non-contact erections test was also drug-free, and occurred