Behavioural Brain Research 283 (2015) 69–77

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Conditioned same-sex partner preference in male rats is facilitated by oxytocin and dopamine: Effect on sexually dimorphic brain nuclei☆

Rodrigo Triana-Del Rio[a,1], Miriam B. Tecamachaltzi-Silvarán[a], Victor X. Díaz-Estrada[a], Deissy Herrera-Covarrubias[a], Aleph A. Corona-Morales[b], James G. Pfaus[c], Genaro A. Coria-Avila[a,*]

[a] Centro de Investigaciones Cerebrales, Universidad Veracruzana, Avenida Luis Castelazo s/n Col. Industrial Ánimas, C. P. 91190, Xalapa, Veracruz, Mexico
[b] Laboratorio de Investigación Genómica y Fisiológica, Facultad de Nutrición, Universidad Veracruzana, Xalapa, Veracruz, Mexico
[c] CSBN/Psychology, Concordia University, Montreal, QC, Canada

HIGHLIGHTS

- We assessed the development of conditioned same-sex preference in rats.
- Males received a D2 agonist or oxytocin and cohabited with another male.
- In a drug-free test males chose between the male and a sexually receptive female.
- Conditioned preference for a male was observed with social and sexual behaviors.
- Same-sex preference did not correlate with changes in sexually dimorphic nuclei.

ARTICLE INFO

Article history:
Received 8 December 2014
Received in revised form 6 January 2015
Accepted 10 January 2015
Available online 16 January 2015

Keywords:
Conditioning
Homosexual
Partner preference
Sex
Quinpirole

ABSTRACT

Conditioned same-sex partner preference can develop in male rats that undergo cohabitation under the effects of quinpirole (QNP, D2 agonist). Herein, we assessed the development of conditioned same-sex social/sexual preference in males that received either nothing, saline, QNP, oxytocin (OT), or QNP + OT during cohabitation with another male (+) or single-caged (−). This resulted in the following groups: (1) Intact−, (2) Saline+, (3) QNP−, (4) OT−, (5) QNP+, (6) OT+ and (7) QNP/OT+. Cohabitation occurred during 24 h in a clean cage 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. 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 males from groups Intact−, Saline+, QNP− and OT− displayed a clear preference for the female (opposite-sex), whereas groups QNP+, OT+ and QNP/OT+ displayed socio/sexual preference for the male partner (same-sex). In Experiment 2, the brains were processed for Nissl dye and the area size of two sexually dimorphic nuclei (SDN-POA and SON) was compared between groups. Males from groups OT−, OT+ and QNP/OT+ expressed a smaller SDN-POA and groups QNP+ and QNP/OT+ expressed a larger SON. Accordingly, conditioned same-sex social/sexual partner preference can develop during cohabitation under enhanced D2 or OT activity but such preference does not depend on the area size of those sexually dimorphic nuclei.

☆ 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).

* Corresponding author. Tel.: +52 228 8418900x13609.
E-mail addresses: rodrigo_elcid16@hotmail.com (R. Triana-Del Rio), anaconda13713@gmail.com (M.B. Tecamachaltzi-Silvarán), leo_victor1989@hotmail.com (V.X. Díaz-Estrada), dherrera@uv.mx (D. Herrera-Covarrubias), alecorona@uv.mx (A.A. Corona-Morales), jim.pfaus@concordia.ca (J.G. Pfaus), gcoria@uv.mx, coria75@yahoo.com (G.A. Coria-Avila).
[1] Present address: Centre de Neurosciences Psychiatriques. CHUV-Université de Lausanne, Lausanne, Switzerland.

http://dx.doi.org/10.1016/j.bbr.2015.01.019
0166-4328/

1. Introduction

Understanding the role of Pavlovian conditioning on brain function and its effects on the development of learned partner preferences is of great relevance for the behavioral neurosciences (for review see [1–5]). Learned preferences may develop when a conditioned stimulus (CS) is associated in contingency with an unconditioned stimulus (UCS) that functions as reinforcer. Consequently, an individual may display preference for a partner that bears that specific CS. There are many types of reinforcers, and depending on critical periods of development they may be more or less effective and may be different for males and females. For example, stimuli associated with nurture and juvenile play are rewarding only during periods of early development and can facilitate the crystalization of partner preferences that will be displayed after puberty [6–10]. In adulthood, other stimuli such as sexual reward [2,11–14], cohabitation [15], mild stress [16] or even pharmacological manipulations [16–19] may function as reinforcers.

Partner preferences are formed, strengthened, or weakened, because reinforcers exert an effect on the dynamics of certain brain neurotransmitters such as dopamine [20,21], opioids [22–25], oxytocin (OT) and vasopressin [26–31]. These neurotransmitters modulate attention, prediction, expectation, reward, and trust [20,32–35], which may be considered the emotional substrates for partner preference. Therefore, any stimulus that affects the dynamics of these neurotransmitters may increase or decrease the probability for the development or maintenance of a conditioned preference. For instance, original studies by Wang and colleagues showed that enhancing the activity of the dopaminergic D2-type receptor with the agonist quinpirole (QNP) resulted in a higher probability for female prairie voles (Michrotus ochrogaster) to develop a strong heterosexual preference for a male they cohabited with (without mating) during a period of 6 h [36], and similar results have been shown if prairie voles are treated with OT during cohabitation [26,30,37]. Indeed, an important synergism between D2 and OT activity has been described in the development of social attachments and pair bonds in prairie voles and other species [31,37–42].

Interestingly, recent studies from our laboratory have shown a role of conditioning on the development of same-sex social partner preferences. If repeated cohabitation occurs between two male rats and one of them is treated with QNP, a strong conditioned same-sex socio/sexual preference develops towards the cagemate in a subsequent drug-free preference test [17,18]. In our studies, same-sex partner preference occurs many days after the last pharmacological manipulation, and it is inferred when a male displays more social and sexual behaviors towards the male he previously cohabited with even if a sexually receptive female rat is concurrently available as a partner choice [17,18]. For example, conditioned males from previous studies preferred a male over a female as he spent more time together (70% of the time), displayed more visits, body contacts, and genital investigations. In addition, they displayed what appeared as female-like solicitations, which are an indication of female sexual proceptivity. In separate tests of non-contact erections, conditioned males displayed more when exposed to a male partner relative to a female partner behind a screen, compared to unconditioned saline-treated males who displayed more non-contact erections on response to females. Given that evidence, it has been suggested that under the effects of the appropriate brain dynamics conditioning is so powerful that it can override presumably innate heterosexual preferences [3]. However, we do not know yet what brain changes are involved during the conditioning process from opposite- to same-sex socio/sexual preference.

In the last 50 years many studies have supported the dogma that emerged from the organizational hypothesis [43] which indicates that when a developing brain is exposed to gonadal hormones some neural substrates become organized and are selectively activated later in adulthood to facilitate the expression of sexually differentiated sexual behavior. Among all brain substrates that are perinatally organized, the sexually dimorphic nucleus of the medial preoptic area (SDN-POA) is perhaps the most studied. It is formed by a group of Nissl dense cells and it is 5–7 times larger in males than females as result of the exposure to testosterone during the first postnatal days [44–46]. Indeed, the lack of testosterone in males during that critical period results in a smaller female-like SDN-POA [45]. Some studies in experimental animals have suggested that the dimorphism of the SDN-POA (or homologous areas in humans) correlates with sexual preference and therefore may be part of a neurocircuitry that is organized perinatally to direct sexual partner preference in adulthood [44–50]. Interestingly, there is evidence indicating that its size can be modified in adult rodents by exposure to the opposite-sex hormones or by sexual experience [51,52], which indicates some flexibility in its organization beyond the critical period. Another nucleus reported as sexually dimorphic is the supraoptic nucleus of the hypothalamus (SON), which is also larger in males than females and contains OT and vasopressin neurons [53], which are required in the formation of partner preferences. Thus, based on our previous findings with the D2 agonist QNP, in Experiment 1 we hypothesized that cohabitation between males under the effects of OT or QNP + OT would also result in the development of same-sex preference. In addition, we wanted to confirm that neither cohabitation alone, nor the drugs themselves induce same-sex preference. Thus, we also evaluated partner preference in males that cohabited under the effects of saline, and in males that received QNP or OT without cohabitation with another male. In Experiment 2, we hypothesized that conditioned males that developed a same-sex preference would expressed a reduction in the size of the SDN-POA and SON.

2. Material and methods

2.1. Experiment 1. Conditioned partner preference

2.1.1. Subjects

All efforts were made to minimize animal suffering, and to reduce the number of animals used. Seventy Wistar (W) male rats were used as experimental subjects to be conditioned and twenty-five males and twenty-five females were used as stimulus animals. All of them were purchased from a certified animal supplier (Rismart®) 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 commercial aspen chip (Rismart®), whereas experimental rats were housed individually during two weeks before the start of the study. All rats 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, Mexico. Water and rodent feed (Rismart®) were provided *ad libitum*.

2.1.2. Conditioned same-sex partner preference

2.1.2.1. Sexual training and surgery. As in our previous studies [17,18] males that functioned as stimulus received at least 10 trials of multi-ejaculatory sexual experience with receptive females prior to the start of the experiment, whereas experimental males were sexually naïve. In those previous studies we observed that cohabitation between two sexually naive males results in a strong same-sex socia preference, but less robust sexual preference. Stimulus females were used in the final partner preference test. They were ovariectomized (OVX) and primed fully with subcutaneous (s.c.) injections of estradiol benzoate (10 μg) 48 h and