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Same-sex cohabitation under the effects of quinpirole induces a conditioned socio-sexual partner preference in males, but not in female rats ☆

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ABSTRACT

The effects of the dopamine D2-type receptor agonist quinpirole (QNP) were examined on the development of conditioned same-sex partner preference induced by cohabitation in rats. In Experiment 1, males received either saline or QNP (1.25 mg/kg) and cohabited during three trials with almond-scented stimulus males that were sexually naïve. In Experiment 2, males received six trials, and in Experiment 3 received three trials with sexually expert stimulus males. During a final drug-free preference test, males chose between the familiar or a novel male partner. In Experiments 1, 2 and 3 only QNP-treated males displayed a social preference for the familiar male, observed with more time spent together. In Experiment 3 males also displayed a sexual preference observed with more non-contact erections when were exposed to their male partner. In Experiment 4 we tested the effects on OVX, E+P primed females that received 1 systemic injection of either saline or QNP during three conditioning trials. In Experiment 5, females received 2 injections 12-h apart during each trial. Results indicated that both saline and QNP-treated females failed to develop partner preference. These data demonstrate that enhanced D2-type receptor activity during cohabitation facilitates the development of conditioned same-sex partner preference in males, but not in female rats. We discuss the implications for same-sex partner preferences.

1. Introduction

The biological bases of homosexual partner preference have been widely discussed in humans and animal models, including the genetic, neuroendocrine and neuroanatomical differences between homosexual and heterosexual individuals (Gulia and Mallick, 2010; LeVay, 1991; Roselli et al., 2011; Savic et al., 2005; Swaab et al., 1995; Weinrich, 1982). However, to the best of our knowledge, there is no report about the effects of learning on the development of homosexual partner preferences.

Many reports on laboratory animal models indicate that learning can shape heterosexual partner preferences via Pavlovian conditioning (Coria-Avila et al., 2008b; Kippin and Pfaus, 2001a, b; Pfaus et al., 2001; Pfaus et al., 2003). Hence, a mate can be seen as a conjunction of multiple stimuli. Some of them are unconditioned stimuli (UCS), which trigger unconditioned responses (UCRs), but many others are not. Ineffective natural stimuli, may become associated with rewarding UCSs through experience, and in turn, may be able to elicit conditioned responses (CRs) that facilitate motivation for a partner in future encounters. This can also occur with originally neutral stimuli. Furthermore, certain patterns in an environment or context can be conditioned to sexually relevant UCSs and in that way become able to elicit CRs.

Conditioning of partner-related stimuli may occur during the early postnatal phase via imprinting, and after puberty, during an animal's first sexual encounters. Imprinting commonly occurs when certain stimuli are sensed during critical periods of life and become associated with innate rewards (maternal care, nutrient intake, etc.) (Batenson, 1978; Fillion and Blass, 1986). Thus, when an individual reaches puberty may prefer partners that bear imprinted stimuli. However, the consequences of the first sexual encounters may also shape future partner preferences when new stimuli are associated with other types of reward. Male rats, for example, develop a conditioned partner preference for females bearing an odor, such as almond or lemon (CS), that was previously associated with the sexual reward state (UCS) induced by ejaculation (Kippin and Pfaus, 2001a, b). Similarly, female rats learn to prefer males that bear cues previously paired with sexual

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reward (Coria-Avila et al., 2006; Coria-Avila et al., 2005; Parada et al., 2011). In monogamous species, like prairie voles, copulation or prolonged periods of cohabitation also facilitate the conditioning of partner preference, commonly referred to as pair bonding (Williams et al., 1992).

In most studies with rats and voles, partner preference and pair bonds are inferred when an individual spends more time in close contact with another, displays directed courtship behavior and selective copulation. It has been shown that manipulations of the dopaminergic system can either facilitate or disrupt those behaviors and therefore is said to modulate partner preferences. For example, female rats that receive a systemic low dose of the dopamine (DA) antagonist flupenthixol during several conditioning trials of paced copulation, fail to develop a conditioned preference for a male scented with the conditioned odor in a final drug-free test (Coria-Avila et al., 2008a). By contrast, low doses of the DA agonist apomorphine during one conditioning trial of cohabitation facilitate the formation of partner preference in monogamous voles (Aragona et al., 2003; Gingrich et al., 2000). More studies have shown that pair bonding is facilitated if cohabitation occurs under the effects of the specific DA D2-type receptor agonist quinpirole (QNP) (Aragona et al., 2006; Wang et al., 1999) injected systemically or in the nucleus accumbens (NAcc), but not if cohabitation occurs under the effects of a D1-type receptor agonist (Aragona et al., 2006). In addition, antagonists for the D2-type receptor (i.e. eticlopride) disrupt the formation of partner preference after mating, or after cohabitation in voles (Gingrich et al., 2000).

Taken together, the existing data in rodents indicate that conditioned preference for a particular partner (or his/her cues) may develop as a result of the contingency between CS and UCS during enhanced activity of the DA D2-type receptors. Thus, in the present study we assessed the possibility cohabitation with an individual of the same sex under the effects of QNP would induce an olfactory conditioned homosexual partner preference in rats.

2. General methods

2.1. Subjects

Wistar (W) male and female rats were used (250–300 g). Males were purchased from Harlan, Mexico, and females were locally bred in our colony room. For the purpose of our study they were randomly categorized as either stimulus or experimental. Stimulus rats were housed in groups of 5, whereas experimental rats were housed individually (except during conditioning trials when they were exposed to cohabitation). All of them were kept in plexiglas cages with a thin layer of wood shaving, maintained in at room temperature on a normal 12:12 h light/dark cycle (lights off at 20:00 h), at the Instituto de Neuroetología, Universidad Veracruzana. Water and rodent chow (Purina) were provided ad libitum.

The study consisted of five separate experiments (3 on males, 2 on females) that tested conditioned same-sex socio/sexual partner preference as a consequence of cohabitation with another individual under the effects of a D2-type agonist (see Table 1).

2.2. Drugs

The dopamine D2-type receptor agonist quinpirole dihydrochloride (QNP) (Sigma; St. Louis, MO) was dissolved in 0.9% physiological saline and injected intraperitoneally in a dose of 1.25 mg/kg (as in Wang, et al., 1999) in a volume of 1 ml/kg 1 min before every conditioning trial. Rats that served as controls were injected with 1 ml/kg of physiological saline 1 min before conditioning.

2.3. Odor conditioning

Every conditioning trial lasted for 24 h (started at 20:00 h and finished at 20:00 h of the following day), and occurred every 4 days. During conditioning, experimental rats received either QNP or saline and 1 min later were placed in a medium size plexiglas cage (20 cm × 30 cm × 45 cm) for cohabitation during 24 h with a matched stimulus rat of the same sex (referred to as the familiar partner). The familiar partner 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 number of conditioning trials for every experiment is shown in Table 1.

2.4. Sexual training and surgery

Stimulus males that required sexual experience received at least 10 trials of sexual training with ovariectomized, hormone-primed females before they served as stimulus (Experiment 3). When ovariectomy was required (Experiments 3, 4 and 5), females were anesthetized with a mixture of 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 ovariectomized 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 females were given a week of post-surgical recovery. Sexual receptivity was induced in all ovariectomized females by subcutaneous injections of estradiol benzoate (10 μg) 48 h and progesterone (500 μg) 4 h before each test.

2.5. Partner preference test

Same-sex partner preference tests occurred four days after the final conditioning trial. Peak plasma concentrations of QNP are

Table 1
Experimental design of the study.

Experiment	Experimental rats	Number of trials	N	Stimulus rats were	QNP or saline *injections per* trial	Preference tests
1	Males	3	QNP = 18 Saline = 18	Sexually naïve	1	First: 4 days after trial 3 Second: 45 days later
2	Males	6	QNP = 8 Saline = 10	Sexually naïve	1	First: 4 days after trial 6 Second: 45 days later
3	Males	3	QNP = 20 Saline = 20	Sexually experienced	1	Non-contact erections First: 4 days after trial 3 Second: 45 days later
4	Females	3	QNP = 10 Saline = 10	OVX, E+P sexually naïve	1	First: 4 days after trial 3
5	Females	3	QNP = 9 Saline = 9	OVX, E+P sexually naïve	2	First: 4 days after trial 3

observed about 15 min after administration, and up to 96% of the drug is recovered in the urine within the following 72 h (Whitaker and Lindstrom, 1987). Consequently, the final preference test was occurred without any drug on board. During the preference test, experimental rats were placed into a three-compartment chamber that had a thin layer of wood shaving. 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 goal compartment (same size as the start compartment) there was the familiar scented stimulus partner (of the same sex), and in the other goal compartment there was a novel unscented partner never seen before (of the same sex). The two stimulus partners wore rodent jackets, connected to a spring wire 20 cm in length, which allowed them to roam within their own chamber, but not beyond. Thus, experimental rats were allowed to interact freely with the two rats that served as stimulus for 20 min.

Partner preference tests were video recorded and scored using the computerized software BOP (behavioral observation program) (Cabilio, 1998). As in previous studies, partner preference was inferred when an individual spent more time in close contact with an individual (Aragona et al., 2003; Carter et al., 1992; DeVries et al., 1996; Lim et al., 2004; Wang and Aragona, 2004; Wang et al., 1999; Young and Wang, 2004). However, we also assessed latency and frequency measures of visits, olfactory investigations (on the back and neck), genital investigations, rough and tumble, mounts. In Experiment 3, we additionally assessed non-contact erections. In Experiments 4 and 5 we assessed female proceptive behaviors such as solicitations (defined as a head-wise orientation to the stimulus female followed by a runaway) and hops and darts. When partner preference occurred (as observed by time together), we carried out a second test 45 days later with the aim to determine whether or not the preference was transitory.

2.6. Statistical analysis

For all the experiments we used a 2 × 2 (odor × drug) analysis of variance (ANOVA) to determine main effects of drug (saline vs. QNP) or odor (scented vs. unscented males) or any interaction between drug and odor, using Statistica Software v. 7.0. Only significant differences were followed by a Fisher's least significant difference (LSD) post hoc test to assess differences between individual means. For the analysis of proportions we used a Fisher's exact test. The level of significance was set at $p<0.05$.

3. Experiment 1

The first experiment tested male homosexual partner preference for a sexually naïve partner after three cohabitation trials under the effects of QNP.

3.1. Method

A total of 36 experimental males were used. Half of the experimental males received QNP (n = 18) and the other half received saline (n = 18) as explained above in Section 2.2. After being injected, all the experimental males were placed to cohabitate with an almond-scented stimulus male (see Section 2.3). The same males formed the couples during the three conditioning trials.

3.2. Results

Table 2 shows the means plus/minus standard errors for all the behaviors assessed during the 20-min test. The ANOVA detected an interaction between drug × odor in the total time spent within the goal compartment $F(1, 34) = 07.69$, $p = 0.009$. The post hoc analysis revealed that only QNP-treated males spent more time within the compartment of the scented (familiar) male. Furthermore, there was an interaction between drug × odor in time spent together $F(1, 34) = 4.50$, $p = 0.041$ (see Fig. 1). The post hoc analysis revealed that only the QNP-treated males spent more time in close contact with the scented male relative to the unscented (novel) male. The frequency of olfactory investigations showed an interaction between drug × odor $F(1, 34) = 4.81$, $p = 0.03$, where only QNP-treated males displayed more olfactory investigations towards scented males. There was also an interaction in the latency for first episode of rough and tumble $F(1, 34) = 5.80$, $p = 0.02$. The post hoc analysis indicated that QNP-treated males displayed shorter latency towards scented, relative to unscented males. For the first genital investigation was an interaction (drug × odor) $F(1, 34) = 4.70$, $p = 0.03$. The post hoc analysis indicated that QNP-treated males displayed their first genital investigation faster towards scented males relative to unscented males.

Table 2
Indicates the mean ± SEM of the different behaviors assessed in Experiment 1. Experimental males (saline or quinpirole-treated) displayed towards the stimulus males (familiar scented vs. novel unscented). Experimental males received three conditioning trials. Stimulus males were sexually naïve.

Behavior of experimental males	Saline group (n = 18)		Quinpirole group (n = 18)	
	Scented (CS+)	Unscented (CS−)	Scented (CS+)	Unscented (CS−)
Social behavior				
First visit latency (in seconds)	29.7 ± 8.7	34.7 ± 9.6	34.6 ± 11	52.3 ± 23.5
Visit frequency	35.7 ± 3.3	35.3 ± 3.7	40.7 ± 1.9	42.1 ± 3.4
Total time within cage with male (s)	349.6 ± 26	326.6 ± 41	491.6 ± 41	402 ± 36
Total time spent together (s)	197.4 ± 23	227.3 ± 21	299.6 ± 26*	205 ± 25
First olfactory investigation latency (s)	36.9 ± 8.3	60.2 ± 19	41.7 ± 12.5	57.4 ± 25
Olfactory investigation frequency	25 ± 4.2	24.3 ± 2.3	37.4 ± 5.2*	22 ± 1.3
Play behavior				
First rough and tumble latency (s)	541.1 ± 113.1	371.6 ± 108	386 ± 101*	827.2 ± 96
Rough and tumble frequency	1.8 ± 0.6	2.2 ± 0.7	4.1 ± 1.4	1.6 ± 0.4
Copulatory behavior				
First genital investigation latency	211.2 ± 49	108.2 ± 20	86.3 ± 29	194.1 ± 69
Genital investigation frequency	6.1 ± 1.8	7.7 ± 1.1	8.7 ± 1.6	6.6 ± 1.9
Hops and darts	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Mounts	0.2 ± 0.2	0.2 ± 0.2	0.7 ± 0.3	0.4 ± 0.2
Intromissions	None	None	None	None
Ejaculations	None	None	None	None
Couples that mounted, number (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

* Significant difference within groups.

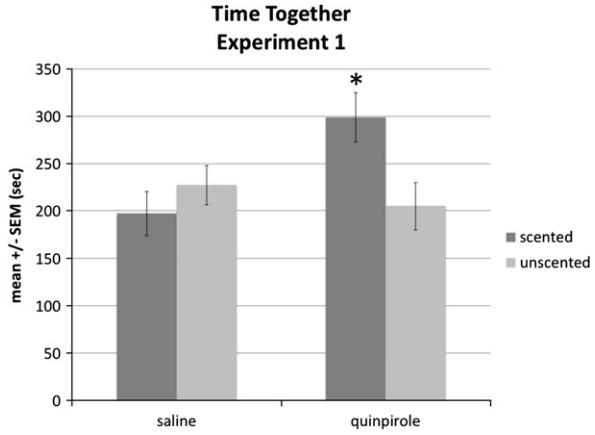

Fig. 1. Mean ± SEM of time spent in close contact between experimental males (saline or quinpirole-treated) and the stimulus males (familiar scented vs. novel unscented) in Experiment 1. Experimental males received three conditioning trials. Stimulus males were sexually naïve. * = $p<0.05$ within groups.

The ANOVA failed to detect main effects or interaction (drug × odor) in the following behaviors: first visit latency, visit frequency, first olfactory investigation latency, and rough and tumble frequency, or genital investigations. Specific copulatory behaviors such as mounts, intromissions or ejaculations were absent between the experimental male with the stimulus males (see Table 2 for further details). Forty-five days later, a second partner preference test failed to detect significant differences in time together or any other behavior.

4. Experiment 2

The second experiment tested male homosexual partner preference for a sexually naïve partner after six cohabitation trials under the effects of QNP.

4.1. Method

A total of 18 experimental males were used. Some experimental males (n = 8) received QNP and the rest received saline (n = 10) as explained above in Section 2.2. After being injected, all the experimental males were placed to cohabitate with an almond-scented stimulus male (see Section 2.3). The same males formed the couples during the three conditioning trials.

4.2. Results

Table 3 shows the means plus/minus standard errors for all the behaviors assessed during the 20-min test. There was an interaction (drug × odor) in the total time spent within the goal compartment $F(1, 16) = 9.38$, $P = 0.008$. The post hoc analysis revealed that only QNP-treated males spent more time within the goal compartment of the scented familiar male, relative to the unscented, and this did not occur in the saline group. There was a main effect of odor in time spent together $F(1, 16) = 10.62$, $P = 0.005$ (see Fig. 2). The post hoc analysis showed that scented familiar males were preferred in both groups (saline and QNP), indicating that cohabitation for six trials without QNP (as in saline group) was also sufficient to induce a partner preference.

There was an interaction (drug × odor) in the frequency of olfactory investigations $F(1, 16) = 4.81$, $p = 0.03$. The post hoc test indicated that QNP-treated males displayed more investigations relative to every other group. There was an interaction (drug × odor) in genital investigation frequency $F(1, 16) = 7.17$, $p = 0.01$. The post hoc analysis revealed that scented males received more genital investigations in the QNP group, but unscented males received more genital investigations in the saline group. There was an interaction (drug × odor) in first rough and tumble latency $F(1, 16) = 5.80$, $p = 0.02$. However, the post hoc analysis only detected differences between scented males in the QNP group vs. unscented males in the saline group.

The ANOVA failed to detect main effects or interactions (drug × odor) in the following behaviors: first visit latency, visit frequency, first olfactory investigation latency, and rough and tumble frequency. Only a small (non-significant) proportion of couples engaged in mounting behavior with the stimulus males. Intromissions and ejaculations were not present in any couple (see Table 3). Forty-five days later, a second partner preference test failed to detect significant differences in time together or any other behavior.

5. Experiment 3

The third experiment tested male homosexual partner preference for a sexually experienced partner after three cohabitation trials under the effects of QNP. In addition, we assessed the number of non-

Table 3
Indicates the mean ± SEM of the different behaviors assessed in Experiment 2. Experimental males (saline or quinpirole-treated) displayed towards the stimulus males (familiar scented vs. novel unscented). Experimental males received six conditioning trials. Stimulus males were sexually naïve.

Behavior of experimental males	Saline group (n = 10)		Quinpirole group (n = 8)	
	Scented (CS+)	Unscented (CS−)	Scented (CS+)	Unscented (CS−)
Social behavior				
First visit latency (s)	15.4 ± 5.7	164.7 ± 105	25.9 ± 10	35.5 ± 20
Visit frequency	27 ± 5.5	30.4 ± 8.9	28.2 ± 4.0	27 ± 3.6
Total time within cage with male (s)	325.2 ± 3.4	202.9 ± 2.4	345.6 ± 5.4*	230.65 ± 1.5
Total time spent together (s)	300.8 ± 30*	172.5 ± 39	304.4 ± 22*	209 ± 33
First olfactory investigation latency (s)	28.12 ± 9.6	169.3 ± 103	30.8 ± 3.9	38.6 ± 19
Olfactory investigation frequency	24.4 ± 1.5	30.4 ± 4	41.3 ± 3.4*	21.8 ± 3.4
Play behavior				
First rough and tumble latency (s)	424.5 ± 69*	108.7 ± 16	308.9 ± 57*	526.2 ± 166
Rough and tumble frequency	1.6 ± 0.8	6.2 ± 3.1	1.8 ± 0.7	2.2 ± 0.8
Copulatory behavior				
First genital investigation latency	281.9 ± 70	209.8 ± 144	169.8 ± 48	188.9 ± 65
Genital investigation frequency	1 ± 0.7*	8 ± 2.8	11 ± 2.6*	4 ± 0.5
Hops and darts	0.2 ± 0.2	0.6 ± 0.4	1.75 ± 0.7	0.25 ± 0.2
Mounts	1.4 ± 1.4	None	None	None
Intromissions	None	None	None	None
Ejaculations	None	None	None	None
Couples that mounted, number (%)	1 (20%)	2 (40%)	1 (20%)	2 (40%)

* Significant difference within groups.

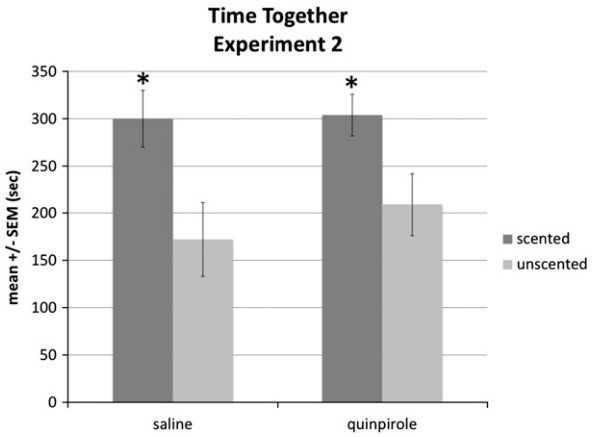

Fig. 2. Mean ± SEM of time spent in close contact between experimental males (saline or quinpirole-treated) and the stimulus males (familiar scented vs. novel unscented) in Experiment 2. Experimental males received six conditioning trials. Stimulus males were sexually naïve. * = $p<0.05$ within groups.

contact erections when experimental and stimulus males were presented behind a wire mesh that allowed olfactory, visual and acoustic stimulation, but not direct contact.

5.1. Method

A total of 40 experimental males were used. Half of the experimental males received QNP (n = 20) and the other half received saline (n = 20) as explained above in Section 2.2. After being injected, all the experimental males were placed to cohabitate with an almond-scented stimulus male (see Section 2.3). The same males formed the couples during the three conditioning trials.

5.2. Results

Table 4 shows the means plus/minus standard errors for all the behaviors assessed during the 20-min test. There was an interaction (drug × odor) in the total time spent within the goal compartments $F(1, 38) = 7.9$, $P = 0.01$. The post hoc analysis revealed that only QNP-treated males visited more time the scented familiar males. There was also an interaction in time spent together $F(1, 38) = 12.7$, $P = 0.002$ (see Fig. 3a). Only QNP-treated males spent more time in close contact with the scented familiar male. There was an interaction in olfactory investigation frequency $F(1, 38) = 1.5$, $p = 0.05$. The post hoc analysis indicated that only QNP-treated males display more olfactory investigation towards scented familiar males. There was an interaction in genital investigation frequency $F(1, 38) = 7.1$, $p = 0.01$. The post hoc analysis revealed that only QNP-treated males displayed more genital investigations towards the scented familiar male. There was an interaction in first rough and tumble latency $F(1, 38) = 5.80$, $p = 0.02$. The post hoc analysis indicated that only QNP-treated males displayed shorter latencies towards scented familiar males.

The ANOVA failed to detect main effects or interactions (drug × odor) in other behaviors such as: first visit latency, visit frequency, first olfactory investigation latency, and rough and tumble frequency. However, a Fisher's exact test revealed a trend ($p = 0.07$) to significance in the proportion of male couples that engaged in mounting behavior in the QNP group vs. saline (Fig. 3b). Intromissions and ejaculations were not present in any couple (see Table 4).

5.3. Non-contact erections

Just prior to the first partner preference test we assessed the frequency of non-contact erections in half of males. The test was drug-free, and occurred in a chamber divided by a wire mesh that allowed visual, olfactory and auditory stimulation, but prevented direct contact between experimental or stimulus males. A transparent

Table 4
Indicates the mean ± SEM of the different behaviors assessed in Experiment 3. Experimental males (saline or quinpirole-treated) displayed towards the stimulus males (familiar scented vs. novel unscented). Experimental males received three conditioning trials. Stimulus males were sexually experts. Different superscript letters in non-contact erections indicate statistical difference.

Behavior of experimental males	Saline group (n = 20)		Quinpirole group (n = 20)	
	Scented (CS+)	Unscented (CS−)	Scented (CS+)	Unscented (CS−)
Social behavior				
First visit latency (s)	39.45 ± 8.2*	66.74 ± 22	43 ± 4.5*	88.5 ± 5.3
Visit frequency	22.2 ± 2.6	21.5 ± 2	21.1 ± 2	19.4 ± 2.2
Total time within cage with male (s)	300.1 ± 25.3	342.4 ± 41	511.7 ± 47.3	260.6 ± 29
Total time spent together (s)	199.4 ± 19.4	209.2 ± 28	380 ± 48.9*	169 ± 13
First olfactory investigation latency (s)	42.7 ± 15.8	70.84 ± 23	46.1 ± 15.6	96 ± 14.6
Olfactory investigation frequency	28.6 ± 4.9	25.8 ± 5.2	48.5 ± 8.7*	29.8 ± 5.5
Play behavior				
First rough and tumble latency (s)	406.5 ± 59*	624.7 ± 28	201.8 ± 73*	593 ± 53
Rough and tumble frequency	1.9 ± 0.7	1.6 ± 0.6	3.5 ± 1.4	1.4 ± 0.7
Copulatory behavior				
First genital investigation latency	353.7 ± 87	128.5 ± 25.5	128 ± 29	148 ± 24
Genital investigation frequency	4.9 ± 1.3*	12.7 ± 2.2	17.7 ± 2.8*	10.2 ± 5.1
Hops and darts	0	0	0.1 ± 0.1	0
Mounts	0	0.5 ± 0.5	0.8 ± 0.8	0
Intromissions	None	None	None	None
Ejaculations	None	None	None	None
Couples that mounted, number (%)	1 (10%)	1 (10%)	5 (50%)	0 (0%)
Non-contact erection test (20 min)	Saline		Quinpirole	
	Experimental	Stimulus	Experimental	Stimulus
Non-contact erections frequency by	0.9 ± 0.2[a]	1.9 ± 1.2[a]	5.2 ± 0.5[b]	4.4 ± 0.6[b]
Grooming frequency by	3.8 ± 0.4*	5 ± 0.5	9.1 ± 2.9	9.26 ± 1.4
Olfactory investigations through the wire mesh	5 ± 0.56	6.3 ± 0.7	13.8 ± 0.3	9.7 ± 1.4

* Significant difference within groups.

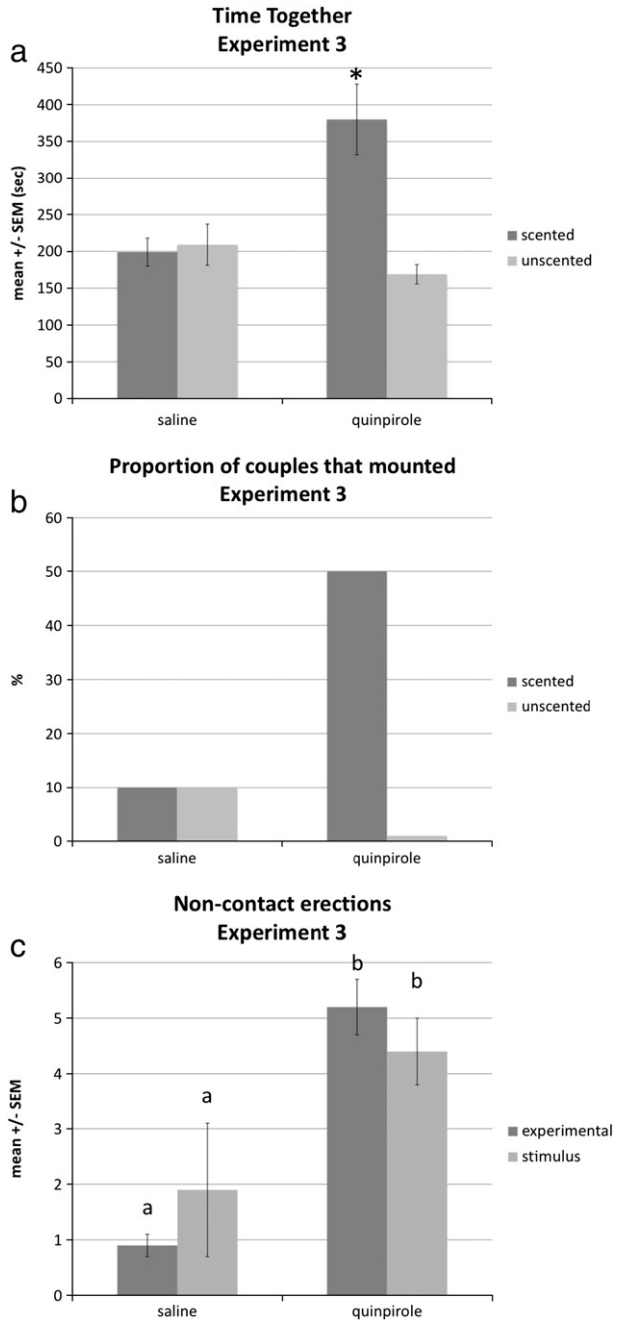

Fig. 3. a. Mean ± SEM of time spent in close contact between experimental males (saline or quinpirole-treated) and the stimulus males (familiar scented vs. novel unscented) in Experiment 3. Experimental males received three conditioning trials. Stimulus males were sexually experts. * = $p<0.05$ within groups. b. Percentage of couples that displayed mounting behavior in Experiment 3. Couples were formed by experimental males (saline or quinpirole-treated) and their stimulus males (familiar scented vs. novel unscented). Experimental males received three conditioning trials. Stimulus males were sexually experts. Fisher's exact test revealed a trend ($p=0.07$) to significance in the proportion of couples that engaged in mounting behavior towards the familiar male in the QNP group vs. saline. c. Mean ± SEM of non-contact erections displayed by experimental males (saline or quinpirole-treated) and also displayed by the stimulus males (familiar scented vs. novel unscented) when were presented behind a wire mesh that allowed visual, olfactory and auditory contact between them, in Experiment 3. Experimental males received three conditioning trials. Stimulus males were sexually experts. Different letters indicate significant differences.

floor of the chamber and a mirror in a 45° angle allowed us to observe and quantify non-contact erections from the bottom of the chamber (Kelliher et al., 1999). A one-way ANOVA was used to detect differences in the total frequency of erections between QNP and saline individuals. The level of significance was set at $p<0.05$.

5.4. Results

The ANOVA detected significant differences in the number of non-contact erections $F(3, 16)=22.43$, $p=0.001$. The post hoc analysis revealed that QNP-treated males displayed more erections when were exposed to the scented familiar males, relative to saline-treated males exposed to the familiar male. In addition, scented males that served as stimulus displayed more erections when were exposed to their QNP-treated male partner, relative to saline-treated male partners (Fig. 3**c**). With regard to the number of olfactory investigations through the wire mesh, the ANOVA detected significant differences $F(3, 16)=16.24$, $p<0.001$. Post hoc analysis indicated that the couples formed by a QNP-treated male and its stimulus scented male displayed more olfactory investigations between them, relative to the saline-treated couples. Forty-five days later, a second partner preference test failed to detect significant differences in time together or any other behavior, including non-contact erections.

6. Experiment 4

This experiment tested female homosexual partner preference for a sexually naïve partner after three cohabitation trials under the effects of one systemic injection of QNP.

6.1. Method

A total of 20 experimental females were used. Half of the experimental females received QNP ($n=10$) and the other half received saline ($n=10$). One minute after being injected, all the experimental females were placed to cohabitate with a scented stimulus female that was also OVX and sexually naïve (see Section 2.3). The two females were kept as couple during the three conditioning trials.

6.2. Results

Table 5 shows the means plus/minus standard errors for all the behaviors assessed during the 20-min test. The ANOVA failed to detect main effects or interaction (drug × odor) in the following behaviors: total time spent within the goal compartment $F(1, 18)=1.3$, $p=0.24$; visit frequency $F(1, 18)=0.05$, $p=0.8$; frequency of close contacts $F(1, 18)=2.0$, $p=0.15$, time together $F(1, 18)=1.9$, $p=0.37$; genital investigations $F(1, 18)=.07$, $p=0.78$. Furthermore, there were no differences in proceptive sexual behaviors like solicitations $F(1, 18)=0.21$, $p=0.64$, and hosp and darts $F(1, 18)=0.18$, $p=0.67$.

7. Experiment 5

Given that females from Experiment 4 failed to display same-sex partner preference, we assessed the effect of two injections of QNP during the conditioning trials. One injection was given at the beginning of every conditioning trials and a second one 12 h later.

7.1. Method

A total of 18 experimental females were used. Half of the experimental females received QNP ($n=9$) and the other half received saline ($n=9$) as explained above in the drugs section. After being injected, all the experimental females were placed to cohabitate with a scented stimulus female (see Section 2.3). The two females were kept as couple during the three conditioning trials.

Table 5
Indicates the mean ± SEM of the different behaviors assessed in Experiment 4. Experimental females (saline or quinpirole-treated) displayed towards the stimulus females (familiar scented vs. novel unscented). Experimental females received one injection of quinpirole during every conditioning trial. Stimulus females were sexually naïve.

Behavior of experimental females	Saline group (n = 10)		Quinpirole group (n = 10)	
	Scented (CS+)	Unscented (CS−)	Scented (CS+)	Unscented (CS−)
Social behavior				
Total time within cage with female (s)	383 ± 45	359 ± 22	313 ± 25	359 ± 18
Total time spent together (s)	383 ± 29	359 ± 19	174 ± 23	202 ± 19
Visit frequency	14.6 ± 1.9	14.9 ± 1.5	13 ± 1	14.4 ± 1
Close contact frequency	10.4 ± 0.8	8.7 ± 0.7	8.8 ± 0.6	9.3 ± 0.7
Sexual behaviors				
Solicitation frequency	0.5 ± 0.4	0.7 ± 0.2	1.7 ± 0.7	2.9 ± 1.9
Hops and darts	0.0 ± 0.0	0.2 ± 0.13	0.1 ± 0.1	0.4 ± 0.16
Olfactory investigations	1.2 ± 0.3	1.3 ± 0.4	1.8 ± 0.8	2.2 ± 0.5
Mount frequency	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Couples that mounted, number (%)	1 (10%)	1 (10%)	1 (10%)	1 (10%)

7.2. Results

Table 6 shows the means plus/minus standard errors for all the behaviors assessed during the 20-min test. The ANOVA failed to detect main effects or interaction (drug × odor) in the following behaviors: total time spent within the goal compartment $F(1, 16) = 2.1$, $p = 0.15$; visit frequency $F(1, 16) = 0.27$, $p = 0.6$; frequency of close contacts $F(1, 16) = 0.10$, $p = 0.75$, time spent together (time in close contact) $F(1, 16) = 3.3$, $p = 0.1$; genital investigations $F(1, 16) = .53$, $p = 0.46$. Likewise, there were no differences in proceptive sexual behaviors like solicitations $F(1, 16) = 0.34$, $p = 0.56$, and hosp and darts $F(1, 16) = 0.18$, $p = 0.67$.

8. Discussion

8.1. Effects on males

The results of the present study indicate that male, but not female rats, can develop a socio-sexual partner preference for individuals of the same sex as a result of repeated cohabitation under the effects of the D2-type receptor agonist QNP. According to our results, cohabitation between two naïve males results in social preference, whereas cohabitation between a naïve and an experienced male results in sexual preference. Specifically, in Experiments 1 only QNP-treated males developed a conditioned social preference for the familiar scented male after three conditioning trials. Social preference was mainly observed as more time spent together (Fig. 1), although males also displayed shorter latencies for their first bout of rough and tumble, and more olfactory investigations towards the preferred male. In Experiment 2, males treated with saline or QNP also formed a preference for the familiar male (Fig. 2), which indicates that several trials (six) of cohabitation facilitated the formation of a social preference. However, only QNP-treated males spent more time in the same cage, displayed more olfactory investigations and shorter latencies for their first genital investigation, and their first bout of rough and tumble, which may suggest higher levels of motivation towards scented males in the QNP-treated males.

In Experiment 3, males received three conditioning trials and cohabitation occurred with sexually experienced males that served as stimulus. Results indicated that males developed a conditioned socio-sexual preference. It was social because only QNP-treated males spent more time in close contact with the scented familiar male (Fig. 3a), visited them faster, and displayed more olfactory and genital investigations. The preference was also sexual because 50% of the couples formed by a scented stimulus male and a QNP-treated male engaged in mounting behavior. This high proportion of mounting behavior was not observed in couples formed with unscented males, or in the saline group, and was close to be statistically significant ($p = 0.07$) (Fig. 3b). In addition, experimental and stimulus males from the QNP group displayed more non-contact erections (Fig. 3c) when were exposed to each other behind a wire mesh (see Section 8.3), which indicates that were more sexually aroused. Taken together, the results of experiments 1–3 indicate that cohabitation with another male during repeated trials may result in same-sex social preference, which can develop much faster via enhancement of the D2-type receptors activity. Such preference may turn it into a sexual preference depending on the previous sexual experience of the partner. This kind of socio-sexual preference is transitory because 45 days later male rats not longer displayed the preferences.

More than one decade ago, Wang et al. demonstrated that systemic injections of a D2- but not D1-receptor antagonist disrupted heterosexual partner preference following mating in

Table 6
Indicates the mean ± SEM of the different behaviors assessed in Experiment 5. Experimental females (saline or quinpirole-treated) displayed towards the stimulus females (familiar scented vs. novel unscented). Experimental females received two injections of quinpirole.

Behavior of experimental females	Saline group (n = 9)		Quinpirole group (n = 9)	
	Scented (CS+)	Unscented (CS−)	Scented (CS+)	Unscented (CS−)
Social behavior				
Total time within cage with female (s)	387.73 ± 48.24	371.30 ± 26.51	355.01 ± 42.86	453.34 ± 35.74
Total time spent together (s)	205.08 ± 31.86	160.94 ± 18.39	172.77 ± 35.98	248.34 ± 40.98
Visit frequency	14.89 ± 3.04	11.33 ± 1.62	14.22 ± 2.46	13.11 ± 1.98
Close contact frequency	8.56 ± 1.43	7.00 ± 0.58	9.56 ± 1.74	8.78 ± 0.72
Sexual behaviors				
Solicitation frequency	0.67 ± 0.29	0.78 ± 0.40	0.44 ± 0.24	0.22 ± 0.15
Hops and darts	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.2
Olfactory investigations	1.56 ± 0.77	1.00 ± 0.55	1.44 ± 0.53	1.78 ± 0.55
Mounts	0.11 ± 0.11	0.00 ± 0.00	0.22 ± 0.22	0.33 ± 0.24
Couples that mounted, number (%)	2 (20%)	0 (0%)	1 (10%)	1 (10%)

voles, whereas a D2- but not a D1-receptor agonist facilitated partner preference without mating. Wang et al. also showed that D2-type receptor activity in the rostral shell of the NAcc facilitate the formation of partner preference (Aragona et al., 2003; Aragona et al., 2006) and that 2 weeks of cohabitation with a female partner induced an up-regulation of D1-type receptors in bonded males, which may help prevent extra-pair bonds (Wang and Aragona, 2004). Accordingly, it is likely that NAcc D2-type receptors also modulate the same-sex conditioned partner preferences observed in our study. Preference for a partner may be formed with several trials of cohabitation, but DA agonists accelerate the neural process. In addition to DA, it is likely that other neurochemicals already known for their role in heterosexual preference may be also involved. These include oxytocin (OT), vasopressin (AVP), opioids, and corticosteroids (Carter et al., 1992; Coria-Avila et al., 2008a; Coria-Avila et al., 2008c; DeVries et al., 1996; Lim et al., 2004; Liu and Wang, 2003).

8.2. Conditioned sexual motivation

Conditioned odors paired in contingency with sex can activate areas that normally respond to unconditioned odors. For example, Kippin et al. (2003) showed that males exposed to estrous odors responded with more Fos in the accessory olfactory bulb, NAcc shell and core, medial bed nucleus of the stria terminalis (BNSTm), medial amygdala (MeA), medial preoptic area (MPOA), ventromedial hypothalamus (VMH) and ventral tegmental area (VTA). However, almond odor paired with sex induced more Fos in the NAcc core, piriform cortex (Pir Ctx), anterior portion of the lateral hypothalamus (aLH), and basolateral amygdala (BLA). Consequently, the authors concluded that conditioned and pheromonal odors activate similar (i.e. NAcc core) but also independent pathways in the limbic system and hypothalamus. Other studies have shown that exposure to conditioned odors previously paired with copulation can increase the levels of luteinizing hormone and testosterone (Graham and Desjardins, 1980). The increases were similar to those following exposure to estrous odors in naïve males, suggesting that the association with the state of reward induced by copulation makes a neutral odor to become a CS capable of triggering a conditioned neuroendocrine response that prepares the animal for sexual behavior. Accordingly, our experimental male rats displayed socio-sexual preference for males that bore the conditioned odor because of the potential neuroendocrine responses similar to those reported by Kippin et al. (2003) and Graham and Desjardins (1980) which resulted in increased motivation. It is worth noting that DA is inhibitory in the olfactory bulb, acting primarily on presynaptic D2 receptors located in the olfactory nerve terminal (Berkowicz and Trombley, 2000; Brunig et al., 1999; Hsia et al., 1999). It is unlikely that the conditioned partner preference observed in our study was due to inhibition of olfaction in QNP-treated rats because the number of injections was very low, compared to experiments that disrupt olfaction. In addition, QNP-treated rats displayed a selective, but not exclusive preference for olfactory investigations towards their familiar males (Tables 2–4).

8.3. Non-contact erections

QNP-treated males displayed more non-contact erections when were exposed to their familiar male partners. Although previous studies have shown that QNP facilitates erections, it is improbable that this was the case for our rats (Depoortere et al., 2009; Hsieh et al., 2004). Peak plasma concentrations of QNP are observed about 15 min after administration, and up to 96% of the drug is recovered in the urine within the following 72 h (Whitaker and Lindstrom, 1987). Given that the rats were tested for non-contact erections 4 days after the last injection of QNP, it is unlikely that the drug had an effect on those erections. Furthermore, stimulus males (non-injected, but exposed to QNP-treated males) also responded with more non-contact erections (Fig. 3-C). Erections indicate a higher level of sexual arousal, and were much less frequent in the saline-treated males and their familiar male partners. This finding is very interesting, because even if QNP alone did facilitate erections, there is no reason why the stimulus males should have more erections too, and they did. Somehow, both males (stimulus and experimental) were affected by the enhancement of D2-type receptors activity in one of them (experimental). We suggest that both males learned from the experience of being with each other. For the experimental male QNP probably increased motivation, expectation, arousal, and learning during the conditioning trials. Because the stimulus male happened to be there during 24 h he probably functioned as many conditioned stimuli that the experimental male learned to associate with increased levels of motivation and arousal. In the case of the stimulus males (sexually experts), it is likely that their non-contact erections were consequence of sexual arousal as well, especially if perceived a "sexual partner" in the experimental males.

The possible link between conditioned same-sex preference in male rats and homosexuality in men may be very debatable, and therefore our results must be interpreted with caution. For example, our findings indicate that cohabitation with a sexually experienced partner under the effects of a D2-type receptor agonist may result in a transitory homosexual preference. Some reports indicate that human homosexuality may be transitory (Delourmel, 2004; Wittenberg, 1956). However, to the best of our knowledge, there are no studies indicating the effect of drugs on the development of conditioned partner preference in humans.

8.4. Effects on females

Our results indicate that female rats did not develop socio-sexual preference following repeated cohabitation under the effects of QNP at the dose administered here (Tables 5 and 6). One possible explanation for the lack of conditioning involves the interaction of DA and oxytocin (OT). For example, previous studies have shown that female rats can develop conditioned partner preferences for males bearing cues associated with paced copulation (Coria-Avila et al., 2006; Coria-Avila et al., 2005). Becker et al. (2001) demonstrated that levels of DA in the NAcc of females were higher if they were allowed to pace the copulatory contact, or if the males were withdrawn from the copulatory arena by the researcher at the females' preferred intervals, compared to non-paced copulation. During the paced copulation or preferred interval situation, the levels of NAcc DA increased about 50% above the baseline 15 min after the male was introduced, and continued to increase until the end of the test, reaching approximately 250% above baseline. In the case of females that were not allowed to pace copulation, or females that paced but had a vaginal mask that prevented intromission, the levels of NAcc DA never increased significantly above baseline, indicating that paced copulatory intervals are more efficient in increasing NAcc DA only when they are associated with intromissive stimulation (Becker et al., 2001). Interestingly, intromissions during paced copulation also activate Fos within OT neurons in the paraventricular and supraoptic nuclei of the hypothalamus in rats (Coria-Avila, 2007; Flanagan et al., 1993), and blood levels of OT are elevated during copulation in animals (McNeilly and Ducker, 1972; Todd and Lightman, 1986) and after orgasm in humans (Blaicher et al., 1999). Thus, it is possible that DA release during paced copulation has to interact with other neurochemicals such as OT, also released during copulation to facilitate the development of partner preferences. In fact, it is possible that such increase is part of the neurochemical substrate of sexual reward, since administration of OT in rats induces conditioned place preference (Liberzon et al., 1997). If OT fails to increase because of the lack of intromissions, then DA alone may not be sufficient to crystallize

partner preferences, as occurred in our receptive female rats. Indeed, the interaction of DA and OT has been demonstrated in studies on monogamous voles (Wang and Aragona, 2004). For example, female voles have more OT receptors in the NAcc, compared to polygamous voles (Insel and Shapiro, 1992), and OT receptor antagonists block partner preferences induced by D2-type receptor agonists like QNP (Liu and Wang, 2003).

Another possible explanation for the lack of conditioning in females involves the effect of estrogens on DA and OT. There is evidence indicating that treatment with steroid hormones in OVX females readily affects the uptake of DA in the NAcc. In some studies, for example, estrogen priming in OVX female rats resulted in a slight decrease in K(+)-stimulated DA release measured in the NAcc, and this decrease was accompanied by a significant increase in both DA reuptake and DA clearance times (Thompson, 1999; Thompson and Moss, 1994). In addition, recent evidence in female meadow voles (a species that forms pair bonds) indicates that estradiol decreases OT receptor binding in the NAcc (Beery and Zucker, 2010). Some studies also indicate that ovarian hormones can affect perseveration of rats (Fernandez-Guasti et al., 2006; van Hest et al., 1989), which may suggest that sexually receptive females lose interest more quickly than unreceptive females. Thus, given that females in our study received estradiol and progesterone to induce sexual receptivity during conditioning, and male rats did not, it may explain how QNP was not effective in females.

8.5. D1/D2 interaction

Graham and Pfaus (2010) have recently demonstrated that D1- and D2-type receptors agonists have a different role in the display of appetitive sexual behavior in female rats. Female rats that received the injections of the D2-type agonist QNP into the medial preoptic area (mPOA) displayed more proceptive behaviors such as hops and darts; whereas female treated with the D1-type agonist SKF displayed less proceptive behavior. It is interesting that chronic treatment with QNP decreases D2-type receptors in rats (Subramaniam et al., 1992), and therefore might affect proceptivity in females. In addition, other study showed that repeated injections of QNP at a dose of 1.5 mg/kg twice a week for 7 weeks, sensitized female hamsters and enhanced D1-receptor-stimulated adenylate cyclase activity in the striatum (Chester et al., 2006). Thus, although our treatment included only three injections, it would be possible that repeated treatment with QNP decreased D2- and enhanced D1-type receptor activity, blocking the capacity of female rats to display a partner preference. However, this explanation is inconsistent with the male data, in which three, or six conditioning trials under the effects of QNP facilitated same-sex partner preference.

9. Conclusion

The results in this study indicate that cohabitation under the effects of enhanced D2-type receptor activity is sufficient to induce a transitory socio-sexual partner preference in male rats, but not in females. The possible link between the data in rats and homosexual behavior in humans is debatable, but it may also shed some light on the effect of learning and dopamine on partner preference formation. Other neurochemicals such as oxytocin (OT), vasopressin (AVP), opioids, and corticosteroids, may be also involved in the modulation of same-sex partner preference in rodents and humans (Carter et al., 1992; Coria-Avila et al., 2008a; Coria-Avila et al., 2008c; DeVries et al., 1996; Lim et al., 2004; Liu and Wang, 2003).

Conflicts of interest

The authors declare no conflicts of interest.

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References

Aragona BJ, Liu Y, Curtis JT, Stephan FK, Wang Z. A critical role for nucleus accumbens dopamine in partner-preference formation in male prairie voles. J Neurosci 2003;23:3483–90.
Aragona BJ, Liu Y, Yu YJ, Curtis JT, Detwiler JM, Insel TR, et al. Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. Nat Neurosci 2006;9:133–9.
Batenson P. Early experience and sexual preference. In: Hutchinson JB, editor. Biological determinants of sexual behavior. Chichester John Wiley & Sons; 1978. p. 29–53.
Becker JB, Rudick CN, Jenkins WJ. The role of dopamine in the nucleus accumbens and striatum during sexual behavior in the female rat. J Neurosci 2001;21:3236–41.
Beery AK, Zucker I. Oxytocin and same-sex social behavior in female meadow voles. Neuroscience 2010;169:665–73.
Berkowicz DA, Trombley PQ. Dopaminergic modulation at the olfactory nerve synapse. Brain Res 2000;855:90–9.
Blaicher W, Gruber D, Bieglmayer C, Blaicher AM, Knogler W, Huber JC. The role of oxytocin in relation to female sexual arousal. Gynecol Obstet Invest 1999;47:125–6.
Brunig I, Sommer M, Hatt H, Bormann J. Dopamine receptor subtypes modulate olfactory bulb gamma-aminobutyric acid type A receptors. Proc Natl Acad Sci USA 1999;96:2456–60.
Cabilio S. Behavioral observation program. Montreal: Concordia University; 1998.
Carter CS, Williams JR, Witt DM, Insel TR. Oxytocin and social bonding. Ann N Y Acad Sci 1992;652:204–11.
Chester JA, Mullins AJ, Nguyen CH, Watts VJ, Meisel RL. Repeated quinpirole treatments produce neurochemical sensitization and associated behavioral changes in female hamsters. Psychopharmacology (Berl) 2006;188:53–62.
Coria-Avila GA. Behavioral and neural mechanisms of conditioned partner preference in the female rat. Psychology. Montreal, Qc: Concordia University; 2007. p 258.
Coria-Avila GA, Ouimet AJ, Pacheco P, Manzo J, Pfaus JG. Olfactory conditioned partner preference in the female rat. Behav Neurosci 2005;119:716–25.
Coria-Avila GA, Jones SL, Solomon CE, Gavrila AM, Jordan GJ, Pfaus JG. Conditioned partner preference in female rats for strain of male. Physiol Behav 2006;88:529–37.
Coria-Avila GA, Gavrila AM, Boulard B, Charron N, Stanley G, Pfaus JG. Neurochemical basis of conditioned partner preference in the female rat: II. Disruption by flupenthixol. Behav Neurosci 2008a;122:396–406.
Coria-Avila GA, Hernandez-Aguilar ME, Toledo-Cardenas R, Garcia-Hernandez LI, Manzo J, Pacheco P, et al. Biological and neural bases of partner preferences in rodents: models to understand human pair bonds. Rev Neurol 2008b;47:209–14.
Coria-Avila GA, Solomon CE, Vargas EB, Lemme I, Ryan R, Menard S, et al. Neurochemical basis of conditioned partner preference in the female rat: I. Disruption by naloxone. Behav Neurosci 2008c;122:385–95.
Delourmel C. A transitory homosexual passion in the course of an analytic treatment. Int J Psychoanal 2004;85:1401–21.
Depoortere R, Bardin L, Rodrigues M, Abrial E, Aliaga M, Newman-Tancredi A. Penile erection and yawning induced by dopamine D2-like receptor agonists in rats: influence of strain and contribution of dopamine D2, but not D3 and D4 receptors. Behav Pharmacol 2009;20:303–11.
DeVries AC, DeVries MB, Taymans SE, Carter CS. The effects of stress on social preferences are sexually dimorphic in prairie voles. Proc Natl Acad Sci USA 1996;93:11980–4.
Fernandez-Guasti A, Agrati D, Reyes R, Ferreira A. Ovarian steroids counteract serotonergic drugs actions in an animal model of obsessive–compulsive disorder. Psychoneuroendocrinology 2006;31:924–34.
Fillion TJ, Blass EM. Infantile experience with suckling odors determines adult sexual behavior in male rats. Science 1986;231:729–31.
Flanagan LM, Pfaus JG, Pfaff DW, McEwen BS. Induction of FOS immunoreactivity in oxytocin neurons after sexual activity in female rats. Neuroendocrinology 1993;58:352–8.
Gingrich B, Liu Y, Cascio C, Wang Z, Insel TR. Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles (*Microtus ochrogaster*). Behav Neurosci 2000;114:173–83.
Graham JM, Desjardins C. Classical conditioning: induction of luteinizing hormone and testosterone secretion in anticipation of sexual activity. Science 1980;210:1039–41.
Graham MD, Pfaus JG. Differential regulation of female sexual behaviour by dopamine agonists in the medial preoptic area. Pharmacol Biochem Behav 2010;97:284–92.
Gulia KK, Mallick HN. Homosexuality: dilemma in discourse! Indian. J Physiol Pharmacol 2010;54:5–20.
Hsia AY, Vincent JD, Lledo PM. Dopamine depresses synaptic inputs into the olfactory bulb. J Neurophysiol 1999;82:1082–5.
Hsieh GC, Hollingsworth PR, Martino B, Chang R, Terranova MA, O'Neill AB, et al. Central mechanisms regulating penile erection in conscious rats: the dopaminergic systems related to the proerectile effect of apomorphine. J Pharmacol Exp Ther 2004;308:330–8.
Insel TR, Shapiro LE. Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. Proc Natl Acad Sci USA 1992;89:5981–5.

Kelliher KR, Liu YC, Baum MJ, Sachs BD. Neuronal Fos activation in olfactory bulb and forebrain of male rats having erections in the presence of inaccessible estrous females. Neuroscience 1999;92:1025–33.
Kippin TE, Pfaus JG. The development of olfactory conditioned ejaculatory preferences in the male rat. I. Nature of the unconditioned stimulus. Physiol Behav 2001a;73: 457–69.
Kippin TE, Pfaus JG. The nature of the conditioned response mediating olfactory conditioned ejaculatory preference in the male rat. Behav Brain Res 2001b;122: 11–24.
Kippin TE, Cain SW, Pfaus JG. Estrous odors and sexually conditioned neutral odors activate separate neural pathways in the male rat. Neuroscience 2003;117:971–9.
LeVay S. A difference in hypothalamic structure between heterosexual and homosexual men. Science 1991;253:1034–7.
Liberzon I, Trujillo KA, Akil H, Young EA. Motivational properties of oxytocin in the conditioned place preference paradigm. Neuropsychopharmacology 1997;17: 353–9.
Lim MM, Wang Z, Olazabal DE, Ren X, Terwilliger EF, Young LJ. Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene. Nature 2004;429:754–7.
Liu Y, Wang ZX. Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. Neuroscience 2003;121:537–44.
McNeilly AS, Ducker HA. Blood levels of oxytocin in the female goat during coitus and in response to stimuli associated with mating. J Endocrinol 1972;54:399–406.
Parada M, Abdul-Ahad F, Censi S, Sparks L, Pfaus JG. Context alters the ability of clitoral stimulation to induce a sexually-conditioned partner preference in the rat. Horm Behav 2011.
Pfaus JG, Kippin TE, Centeno S. Conditioning and sexual behavior: a review. Horm Behav 2001;40:291–321.
Pfaus JG, Kippin TE, Coria-Avila G. What can animal models tell us about human sexual response? Annu Rev Sex Res 2003;14:1–63.
Roselli CE, Reddy RC, Kaufman KR. The development of male-oriented behavior in rams. Front Neuroendocrinol 2011.
Savic I, Berglund H, Lindstrom P. Brain response to putative pheromones in homosexual men. Proc Natl Acad Sci USA 2005;102:7356–61.
Subramaniam S, Lucki I, McGonigle P. Effects of chronic treatment with selective agonists on the subtypes of dopamine receptors. Brain Res 1992;571:313–22.
Swaab DF, Gooren LJ, Hofman MA. Brain research, gender and sexual orientation. J Homosex 1995;28:283–301.
Thompson TL. Attenuation of dopamine uptake in vivo following priming with estradiol benzoate. Brain Res 1999;834:164–7.
Thompson TL, Moss RL. Estrogen regulation of dopamine release in the nucleus accumbens: genomic- and nongenomic-mediated effects. J Neurochem 1994;62: 1750–6.
Todd K, Lightman SL. Oxytocin release during coitus in male and female rabbits: effect of opiate receptor blockade with naloxone. Psychoneuroendocrinology 1986;11:367–71.
van Hest A, van Haaren F, van de Poll NE. Perseverative responding in male and female Wistar rats: effects of gonadal hormones. Horm Behav 1989;23:57–67.
Wang Z, Aragona BJ. Neurochemical regulation of pair bonding in male prairie voles. Physiol Behav 2004;83:319–28.
Wang Z, Yu G, Cascio C, Liu Y, Gingrich B, Insel TR. Dopamine D2 receptor-mediated regulation of partner preferences in female prairie voles (*Microtus ochrogaster*): a mechanism for pair bonding? Behav Neurosci 1999;113:602–11.
Weinrich JD. Is homosexuality natural? In: Paul W, Weinrich JD, Gonsiorek JC, Hotver ME, editors. Homosexuality: social, psychological and biological issues. Beverley Hills, CA: Sage; 1982.. p 203.
Whitaker NG, Lindstrom TD. Disposition and biotransformation of quinpirole, a new D-2 dopamine agonist antihypertensive agent, in mice, rats, dogs, and monkeys. Drug Metab Dispos 1987;15:107–13.
Williams JR, Catania KC, Carter CS. Development of partner preferences in female prairie voles (*Microtus ochrogaster*): the role of social and sexual experience. Horm Behav 1992;26:339–49.
Wittenberg R. Lesbianism as a transitory solution of the ego. Psychoanal Rev 1956;43: 348–57.
Young LJ, Wang Z. The neurobiology of pair bonding. Nat Neurosci 2004;7:1048–54.