

## Enhanced D2-type receptor activity facilitates the development of conditioned same-sex partner preference in male rats<sup>☆</sup>

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### ABSTRACT

Animal models have shown that the neural bases of social attachment, sexual preference and pair bonds, depend on dopamine D2-type receptor and oxytocin activity. In addition, studies have demonstrated that cohabitation can shape partner preference via conditioning. Herein, we used rats to explore the development of learned same-sex partner preferences in adulthood as a result of cohabitation during enhanced D2 activity. Experimental Wistar males (N = 20), received saline or the D2 agonist (quinpirole) and were allowed to cohabitate during 24 h, with a stimulus male partner that bore almond scent on the back as conditioned stimulus. This was repeated every 4 days, for a total of three trials. Four days later they were drug-free tested for partner preference between the scented male partner and a sexually receptive female. Sexual partner preference was analyzed by measuring frequency and latency for appetitive and consummatory sexual behaviors, as well as non-contact erections. Social preference was also analyzed by measuring the frequency and latency of visits, body contacts and time spent together. Results indicated that only quinpirole-treated males displayed sexual and social preference for the scented male over the sexually receptive female. They spent more time together, displayed more body contacts, more female-like proceptive behaviors, and more non-contact erections. Accordingly, conditioned males appeared to be more sexually aroused and motivated by the known male than by a receptive female. We discuss the implications of this animal model on the formation of learned homosexual partner preferences.

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### 1. Introduction

The extent to which same-sex partner preference is determined by “nurture” as opposed to “nature” is controversial. The “nature” evidence is mainly supported by studies on the neuroanatomical differences in some hypothalamic nuclei 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). The “nurture” evidence is supported by the Exotic Becomes Erotic (EBE) theory proposed by Bem (1996), which suggests that biological variables such as genes or prenatal hormones do not code for sexual orientation per se but for

childhood temperaments. The theory suggests how biological variables might interact with experiential and sociocultural factors to influence an individual's sexual orientation (Bem, 2000), so that experience with forbidden, or “exotic” sexual activities becomes “erotic” through learned associations with states of arousal and pleasure. Moreover, animal models have demonstrated that both place and partner preferences can be conditioned during an animal's first experiences not only with sexual reward (Coria-Avila et al., 2005; Ismail et al., 2009; Kendrick et al., 1998; Kippin and Pfaus, 2001; Paredes and Alonso, 1997; Paredes and Vazquez, 1999; Pfaus et al., 2001), but also with non-sexual reward (Paredes-Ramos et al., 2011; Triana-Del Rio et al., 2011).

Pavlovian learning occurs when a neutral cue gains incentive value after being associated in contingency with an unconditioned stimulus (UCS) that produces a rewarding unconditioned response (UCR). After some repetitions, the neutral cue functions as a predictor of the UCS, and becomes a conditioned stimulus (CS) capable of inducing a conditioned response (CR). For example, rats display heterosexual preference for a partner never seen before if it bears an olfactory CS that predicts reward (either sexual or non-sexual) (Coria-Avila et al., 2006, 2005; Kippin and Pfaus, 2001; Parada et al., 2011; Paredes-Ramos et al., 2011). Such conditioned preferences are highly selective and depend on sensitized motivation that did not exist before learning.

The present study examined how Pavlovian associations may alter the development of partner preference towards that of the same sex,

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and how quinpirole (QNP), a dopamine D2-type receptor agonist facilitates this process. Previous studies have demonstrated that enhancing the activation of D2-type receptors facilitates the development of pair bonds in monogamous rodents without the need of mating (Aragona et al., 2003; Gingrich et al., 2000; Williams et al., 1992). Accordingly, activation of D2-type receptors may be part of the UCS that normally occurs during sex, and consequently, cohabitation without mating under the influence of QNP may facilitate an association with specific salient partner cues (CS). A recent study from our group showed that male rats develop a same-sex sociosexual preference after a process of conditioning with QNP (Triana-Del Rio et al., 2011). Males were treated systemically with QNP or saline, and were allowed to cohabit with the same stimulus male for 24 h once every 4 days for a total of 3 conditioning trials. During all the conditioning trials the stimulus male was scented with almond odor that functioned as an olfactory CS. Four days after the last conditioning trial, the rats were tested without drug treatment for sociosexual preference using a three-compartment chamber. One goal compartment contained the almond-scented stimulus male with whom cohabitation occurred under the effects of QNP. In the other goal compartment there was a novel unscented male. We found that male rats from the QNP group spent more time in close contact with the scented male (70% of the time), performed and received mounts with him (more than observed in controls), and displayed more non-contact erections when presented with the preferred male behind a wire mesh screen (80% more erections than controls). We suggested that enhanced D2-type activity during an extended period of cohabitation was sufficient for males to develop a conditioned sociosexual preference for a same-sex conspecific.

One limitation of our previous study was that socio-sexual preference was tested with two males as potential “partners” (Triana-Del Rio et al., 2011). Moreover, the familiar stimulus male bore the almond odor CS, which likely helped the subject male recognize him, whereas the other male was novel and unscented. It is therefore possible that such same-sex preference would not be expressed by males in the presence of a sexually receptive female. Receptive females represent a cluster of natural cues that function as powerful UCSs, which trigger sexual motivation. Thus, in the present study we asked whether learned same-sex preference in males would be expressed in the presence of a sexually-receptive female. Preference for the male over the female under these conditions would be a stronger indication of a same-sex preference.

## 2. General methods

### 2.1. Subjects

Wistar (W) male rats were used as experimental subjects for the conditioning experiments, and as stimulus males and females. All were bred in our colony and had similar body weights (250–300 g). Stimulus rats were housed by sex in groups of 5 in Plexiglas cages with a thin layer of aspen chip (Rismart), whereas experimental rats were housed individually in cages with the same bedding (except during conditioning trials when they were exposed to cohabitation). 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.

### 2.2. Drugs

Ten males were treated with the dopamine D2-type receptor agonist quinpirole dihydrochloride (QNP) (Sigma; St. Louis, MO). It was dissolved in 0.9% physiological saline and was injected intraperitoneally in a dose of 1.25 mg/kg [as in (Triana-Del Rio et al., 2011; Wang et al., 1999)] in a volume of 1 ml/kg, 1 min before every conditioning trial. Ten rats served as controls and were injected with 1 ml/kg of physiological saline 1 min before conditioning.

### 2.3. Partner conditioning

Every conditioning period lasted 24 h (beginning at 20:00 h and finishing at 20:00 h of the following day), and occurred every 4 days. During conditioning, experimental rats received either QNP or saline 1 min before being placed into a Plexiglas cage (20 cm × 30 cm × 45 cm) for cohabitation during the 24-h conditioning period with a 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 same couple cohabitated during every conditioning trial.

### 2.4. Sexual training and surgery

As in our previous experiment (Triana-Del Rio et al., 2011), stimulus males had received 10 trials of multiejaculatory sexual experience with receptive females prior to the start of the experiment, whereas experimental males were sexually naïve. Stimulus females were ovariectomized (OVX) and primed fully with subcutaneous (sc) injections of estradiol benzoate (10 µg) 48 h and progesterone (500 µg) 4 h before each test. For ovariectomy, 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 was injected ip 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 sc injections of flunixin meglumine (2.5 mg/kg) for analgesia, and enrofloxacin (5 mg/kg) every 24 h to prevent post-surgical bacterial infections. All females were given a week of post-surgical recovery before the experiment.

### 2.5. Partner preference test

Preference was tested as in our previous study (Triana-Del Rio et al., 2011), four days after the final conditioning trial. During 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. One goal compartment (same size as the start compartment) contained the scented male, and the other goal compartment contained an unscented sexually-receptive female. The two stimulus partners (male and female) wore rodent jackets, connected to an elastic 20 cm in length, which allowed them to roam within their own chamber, but not beyond. Thus, experimental males were allowed to interact freely with the two rats that served as stimulus for 20 min.

Preference tests were video recorded and scored using the computerized software BOP (behavioral observation program) (Cabilio, 1998). During the preference test experimental males were able to enter the goal compartments with the scented male and unscented female for interaction. As in previous studies, social partner preference was inferred when a male spent more time in close contact with the stimulus partners (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 sexual preference by measuring latency and frequency of sexual behaviors (Table 1). For example, we scored both latency and frequency of genital investigations, mounts, intromissions and ejaculations that the males displayed during the test. However, we also observed that males from both QNP and saline groups displayed head-wise orientations to the stimulus partners followed by a runaway. We considered that this behavior resembled female solicitations (Erskine, 1989), which normally force the male to chase the female. Consequently, we decided to score those “male solicitations” as a measure of female-like proceptive behavior.

## 2.6. Non-contact erections

We additionally assessed sexual arousal in every male by measuring the frequency of non-contact erections evoked by the presence of the scented male, and compared them with the frequency of non-contact erections evoked by the presence of a sexually receptive female (in two separate and counterbalanced tests). The test lasted for 20 min, and occurred just immediately before the partner preference test (e.g. animals tested for non-contact erections at 12:00 h, were tested for partner preference approximately at 12:20 h). The testing rooms were contiguous, and animals were just moved from one room to another right away. Half of the experimental rats from both groups (saline and QNP) were exposed to the scented male on the first day, and exposed to the receptive female the following day. The other half of experimental rats from both groups were exposed to a sexually receptive female on the first day, and exposed to the scented male the following day. Counterbalancing the groups ruled out the possibility of a preference for the first partner. The non-contact erection test of the second day occurred approximately at the same hour for each rat.

The non-contact erection test was drug-free, and occurred in a chamber with two compartments divided by a wire mesh. In one compartment we placed the experimental male and in the other compartment was the stimulus partner (either male or receptive female). This allowed visual, olfactory and auditory stimulations, but prevented direct contact between the experimental rat and the stimulus partner. The chamber had a transparent floor and a mirror in a 45° angle which allowed us to observe and quantify non-contact erections (Kelliher et al., 1999). The test occurred in a separate room, away from all males and females, to prevent the males from detecting receptive females. In addition, the floor and walls of the chamber were cleaned with water and alcohol after each test to eliminate conspecific

odors. We determined differences in the total frequency of erections between QNP and saline individuals during exposure to a male or a sexually receptive female.

## 2.7. Statistical analysis

We used an analysis of variance (ANOVA) with a generalized linear model (GLM) to determine main effects of drug (saline vs. QNP) or partner (scented males vs. unscented sexually receptive females) or any interaction between drug and partner, using the software JMP v. 6.0.0. (SAS Institute). Frequency data fit within a Poisson distribution, whereas data latencies and time spent together fit within a Gamma distribution. GLM is useful in non-normal distributions with non-equal variance and requires a transformation of data by using logarithms. In accordance to the GLM our results are expressed with Chi-squared ( $\chi^2$ ) statistic and not with the F value (Mangeaud and Videla, 2005). Only significant differences were followed by an LSD post hoc test to assess differences between individual means. The level of significance was set at  $p < 0.05$ .

## 3. Same-sex preference in males

### 3.1. Method

A total of 20 experimental males were used. Half of the experimental males received QNP ( $n = 10$ ) and the other half received saline ( $n = 10$ ) as explained above in the **Drugs** section. After being injected, all the experimental males were allowed to cohabitate with an almond-scented stimulus male (see partner conditioning). The same males were paired during the three conditioning trials.

**Table 1**

Indicates the mean  $\pm$  SEM of the different behaviors assessed to identify homosexual partner preference. Experimental males (saline or quinpirole-treated) chose between two stimulus partners (male vs. sexually-receptive female). Experimental males received three conditioning trials.

Behavior of experimental males	Saline group (n = 10)		Quinpirole group (n = 10)	
	Male (CS +)	Female (CS -)	Male (CS +)	Female (CS -)
<b>Social behavior</b>				
First visit latency (s)	49.32 $\pm$ 11.80	34.44 $\pm$ 6.98	54.2 $\pm$ 12.8	77.50 $\pm$ 15.66
Visit frequency at goal compartment	20.70 $\pm$ 2.74	23.00 $\pm$ 3.17	25.4 $\pm$ 1.3 <sup>a</sup>	19.10 $\pm$ 1.13
Time spent visiting the goal compartment (s)	272.05 $\pm$ 28 <sup>a</sup>	483.10 $\pm$ 28.39	502.9 $\pm$ 42.8 <sup>a</sup>	319.72 $\pm$ 21.84
First contact latency (s)	84.79 $\pm$ 15.70 <sup>a</sup>	45.21 $\pm$ 6.68 <sup>#</sup>	67.7 $\pm$ 15.3 <sup>a</sup>	96.64 $\pm$ 19.41
Body contact frequency	15.40 $\pm$ 2.25 <sup>a</sup>	21.30 $\pm$ 2.10	26.90 $\pm$ 2.24 <sup>a</sup>	11.80 $\pm$ 1.07
Time spent with stimulus animal (s)	94.01 $\pm$ 14.45 <sup>a</sup>	193.70 $\pm$ 24.03	247.3 $\pm$ 28.8 <sup>a</sup>	109.41 $\pm$ 11.99
First olfactory investigation latency (s)	132.29 $\pm$ 29.94	69.82 $\pm$ 26.53	63.8 $\pm$ 14.2 <sup>a</sup>	105.27 $\pm$ 22.93
Olfactory investigation frequency	5.00 $\pm$ 1.09	6.30 $\pm$ 0.82	6.4 $\pm$ 0.6 <sup>a</sup>	4.00 $\pm$ 0.77
<b>Play behavior</b>				
First rough and tumble latency (s)	963.10 $\pm$ 51.21	1194.00 $\pm$ 0.00	603.3 $\pm$ 175.2	0.00 $\pm$ 0.00
Rough and tumble frequency	0.40 $\pm$ 0.22	0.50 $\pm$ 0.50	1.9 $\pm$ 0.9	0.00 $\pm$ 0.00
<b>Non-contact erections test</b>				
Frequency of non-contact erections during exposure to:	0.5 $\pm$ 0.25 <sup>a</sup>	5.5 $\pm$ 0.9	3.4 $\pm$ 0.8 <sup>a</sup>	0.4 $\pm$ 0.20
<b>Copulatory behavior</b>				
Solicitation latency (s)	450.92 $\pm$ 144.85	631.30 $\pm$ 134.87	209.0 $\pm$ 47.2	567.49 $\pm$ 140.39
Solicitation frequency	1.80 $\pm$ 0.81	4.50 $\pm$ 2.07	16.8 $\pm$ 5.7 <sup>a</sup>	2.20 $\pm$ 0.71
First genital investigation latency (s)	238.20 $\pm$ 75.33	108.70 $\pm$ 44.89	84.2 $\pm$ 22.2 <sup>a</sup>	317.77 $\pm$ 124.69
Genital investigation frequency	1.10 $\pm$ 0.38 <sup>a</sup>	5.30 $\pm$ 1.24	6.9 $\pm$ 1.3	4.50 $\pm$ 1.39
Hops and darts latency (s)	810.70 $\pm$ 0.00	718.40 $\pm$ 0.00	484.9 $\pm$ 155.7	1042.90 $\pm$ 0.00
Hops and darts frequency	0.10 $\pm$ 0.10	0.10 $\pm$ 0.10	1.4 $\pm$ 0.7	0.10 $\pm$ 0.10
Female defense latency (s)	842.60 $\pm$ 0.00	0.00 $\pm$ 0.00	401.8 $\pm$ 0.0	0.00 $\pm$ 0.00
Female defense frequency	0.10 $\pm$ 0.10	0.00 $\pm$ 0.00	0.5 $\pm$ 0.5	0.00 $\pm$ 0.00
Mount attempt latency (s)	360.30 $\pm$ 0.00	595.35 $\pm$ 118.65	186.2 $\pm$ 0.0	661.30 $\pm$ 0.00
Mount attempt frequency	0.10 $\pm$ 0.10	0.50 $\pm$ 0.40	0.1 $\pm$ 0.1	0.10 $\pm$ 0.10
Mount latency (s)	728.90 $\pm$ 0.00	687.00 $\pm$ 188.40	0.0 $\pm$ 0.0	617.80 $\pm$ 0.00
Mount frequency	0.10 $\pm$ 0.10	4.00 $\pm$ 2.49	0.0 $\pm$ 0.0	0.50 $\pm$ 0.50
First intromission latency (s)	0.00 $\pm$ 0.00	608.27 $\pm$ 114.18	0.0 $\pm$ 0.0	620.60 $\pm$ 0.00
Intromission frequency	0.00 $\pm$ 0.00	1.00 $\pm$ 0.54	0.0 $\pm$ 0.0	0.20 $\pm$ 0.20
First ejaculation latency (s)	0.00 $\pm$ 0.00	1185.20 $\pm$ 0.00	0.0 $\pm$ 0.0	0.00 $\pm$ 0.00
Ejaculation frequency	0.00 $\pm$ 0.00	0.10 $\pm$ 0.10	0.0 $\pm$ 0.0	0.00 $\pm$ 0.00

<sup>a</sup> Significant difference within groups.

<sup>#</sup> Significant difference with female (CS-) in quinpirole group.

### 3.2. Results

#### 3.2.1. Sexual behaviors

Table 1 shows the means plus/minus standard errors for all the behaviors assessed during the 20-min test. There was an interaction (drug × partner) in the total time spent visiting the goal compartments  $\chi^2(1,38) = 30.21$ ,  $p < 0.01$ . The post hoc analysis revealed that only QNP-treated males spent more time in the goal compartment of the scented males, whereas saline-treated males spent more time in the goal compartment of the sexually receptive female. There was an interaction in frequency of visits  $\chi^2(1,38) = 8.34$ ,  $p < 0.01$ . The post hoc analysis indicated that only QNP-treated males displayed more visits with the scented males. There was an interaction in frequency of body contacts  $\chi^2(1,38) = 58.78$ ,  $p < 0.01$ . The post hoc analysis indicated that QNP-treated males displayed higher frequency of contacts towards scented males. By contrast, saline-treated males displayed higher frequency of contacts with the receptive female (Fig. 1). This pattern was also observed in the time in contact with stimulus animals  $\chi^2(1,38) = 27.6$ ,  $p < 0.01$ . QNP-treated males spent more time with the scented male, whereas saline-treated males spent more time with the receptive female (Fig. 2). There was an interaction in the first olfactory investigation latency  $\chi^2(1,38) = 4.88$ ,  $p = 0.02$ . The post hoc analysis revealed that QNP-treated males displayed shorter latency for the first olfactory investigation towards the scented male, and took longer to investigate receptive females. By contrast, saline-treated males investigated the receptive female much faster. There was also an interaction in the frequency of olfactory investigations  $\chi^2(1,38) = 6.5$ ,  $p < 0.01$ . The post hoc analysis indicated that QNP-treated males displayed a higher frequency of olfactory investigations towards scented males. This pattern was also observed in the frequency of genital investigations  $\chi^2(1,38) = 33.25$ ,  $p < 0.01$ . The post hoc analysis revealed that only saline-treated males displayed more genital investigations towards the receptive female. There was an interaction in genital investigation latency  $\chi^2(1,38) = 9.43$ ,  $p = 0.01$ . The post hoc analysis revealed that QNP-treated males displayed shorter latency to investigate the genitals of the scented male, but took longer time to investigate the receptive female.

We also assessed the frequency of female-like proceptive behaviors, and we found an interaction with solicitations  $\chi^2(1,38) = 80.89$ ,  $p < 0.01$ . The post hoc analysis indicated that QNP-treated males displayed higher frequency of solicitations towards scented males, and solicited only few times towards receptive females. Solicitations were not observed frequently in the saline-treated males (Fig. 3). There was also an interaction in solicitation latency  $\chi^2(1,38) = 6.63$ ,  $p < 0.01$ . The post hoc analysis indicated that QNP-treated males displayed shorter latency to solicit scented males. In addition, we observed

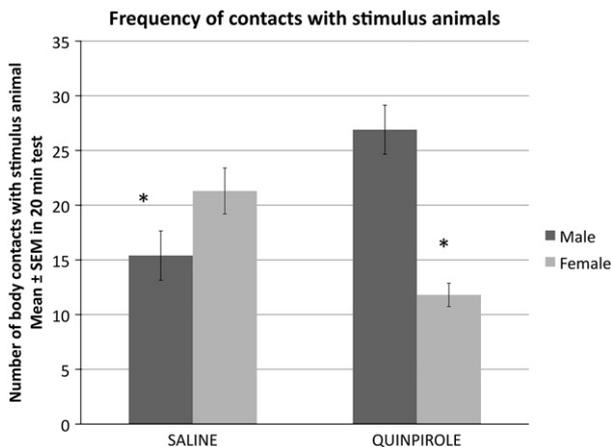


Fig. 1. Mean ± SEM of contact frequency between experimental males (saline or quinpirole-treated) and the stimulus partners (male vs. sexually-receptive female). Experimental males received three conditioning trials. \* =  $p < 0.05$  within groups.

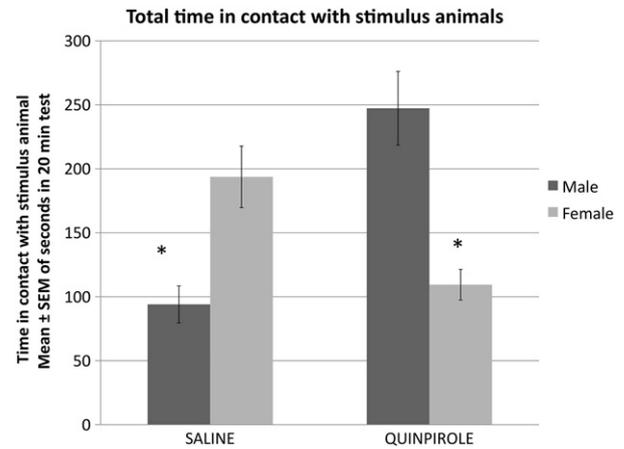


Fig. 2. Mean ± SEM of time spent in close contact between experimental males in Experiment 1 (saline or quinpirole-treated) and the stimulus partners (male vs. sexually-receptive female). Experimental males received three conditioning trials. \* =  $p < 0.05$  within groups.

interesting non-significant trends in the frequency of mounts. For example, saline-treated males displayed mounts towards receptive females, and not towards males. By contrast, QNP-treated males displayed fewer mounts towards receptive females (Fig. 4). For other behaviors the ANOVA failed to detect main effects or interactions (drug × partner). For example, frequency and latency of first visit and rough and tumble, frequency and latency of intromissions, or ejaculations. The low frequency for some behaviors (i.e. ejaculations) may reflect that rats were sexually naïve.

#### 3.2.2. Non-contact erections

The ANOVA detected significant differences in the frequency of non-contact erections  $\chi^2(1,38) = 72.58$ ,  $p < 0.01$ . The post hoc analysis revealed that QNP-treated males displayed a higher frequency of erections when they were exposed to the scented males, relative to the number of erections when they were exposed to sexually receptive females. By contrast, saline-treated males displayed more erections during exposure to the receptive female and displayed only few erections (as expected) during exposure to the male (Fig. 5).

## 4. Discussion

The results of the present study show that male rats can develop a same-sex partner preference in adulthood after a process of conditioning and pharmacological manipulation of D2 activity. We showed

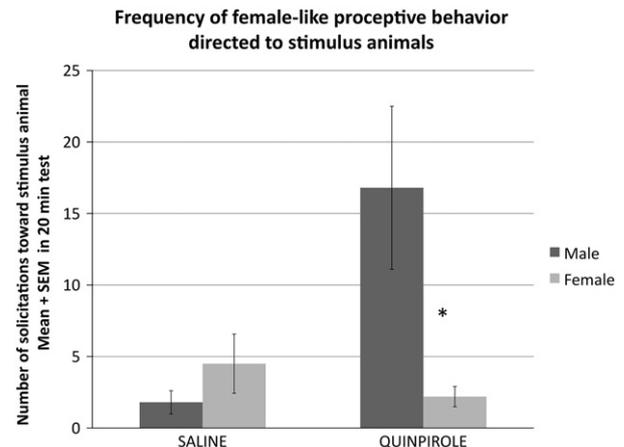


Fig. 3. Mean ± SEM of female-like proceptive behaviors (solicitations) displayed by males (saline or quinpirole-treated) towards the stimulus partners (male vs. sexually-receptive female). \* =  $p < 0.05$  within groups.

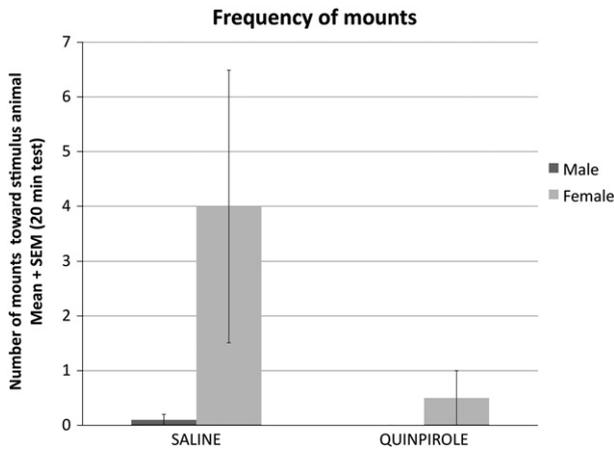


Fig. 4. Mean  $\pm$  SEM of mounts displayed by males (saline or quinpirole-treated) towards the stimulus partners (male vs. sexually-receptive female).

that male rats learned to prefer another male during three conditioning trials of cohabitation under the effects of the D2-type receptor QNP, an effect that did not occur in saline-treated males. In the final partner preference test, males from the QNP group displayed a sociosexual preference for the male partner over the sexually receptive female. Social preference was observed with more visits, shorter visit latency, more contacts (Fig. 1) and more time spent with the male (70% of the time) (Fig. 2). In addition, males from the QNP group displayed more female-like solicitations and directed them towards the male partner (Fig. 3), few QNP males mounted (Fig. 4), or intromitted the receptive female, and none displayed the ejaculation pattern. Males from the QNP group also displayed more non-contact erections evoked by the presence of the male partner, compared to erections evoked by the presence of a sexually receptive female (Fig. 5). Together, these findings suggest that putatively heterosexual adult males modified their socio-sexual preference following a conditioning process under the pharmacological enhancement of D2-type receptor activity.

#### 4.1. The overriding power of learning

The evidence obtained from this study on animal model may be of relevance for understanding the formation of sexual preferences in other species. For instance, these results suggest that an adult male can

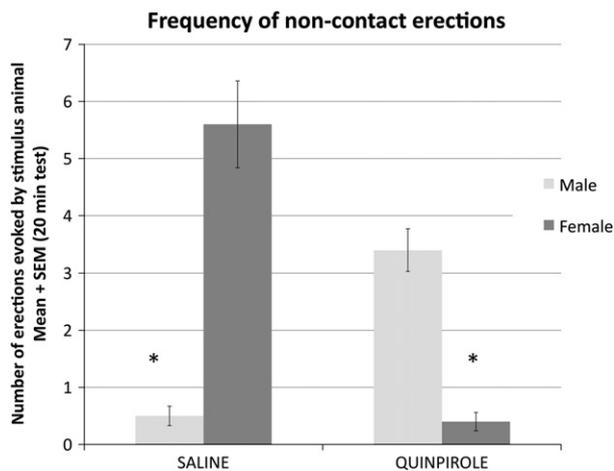


Fig. 5. Mean  $\pm$  SEM of non-contact erections displayed by experimental males (saline or quinpirole-treated) during exposure to a male or a sexually-receptive female presented behind a wire mesh that allowed visual, olfactory and auditory contact between them. \* =  $p < 0.05$  within groups.

weaken his sexual preference for a sexually receptive female, and strengthen his preference for a male after few conditioning trials of same-sex cohabitation under enhanced D2-type activity. The same-sex preference was observed despite the presence of a sexually receptive female, which suggests that the unconditioned preference (i.e. innate) for a sexually receptive female was overridden by the conditioned preference (i.e. learned) for a familiar male. After the process of conditioning the females' cues (UCS) were no longer sufficient to trigger certain aspects of sociosexual motivation, but the male odor (CS) was sufficient.

#### 4.2. Conditioned sexual motivation

The mechanism by which QNP facilitates the formation of same-sex preference during cohabitation is likely related to the findings of Wang and colleagues (Aragona et al., 2006; Wang et al., 1999). Those studies showed that systemic injections of a D2- but not D1-receptor antagonist disrupted heterosexual partner preference following mating in adult monogamous voles, whereas a D2- but not a D1-receptor agonist facilitated partner preference without mating. They also showed that D2-type receptor activity in the rostral shell of the nucleus accumbens (NAcc) facilitated the formation of partner preference in adults. One speculation based in the vole data is that NAcc D2-type receptor activity may also modulate the same-sex conditioned partner preference observed in our study with rats. However, further tests are necessary to support or reject this idea. It is possible that preference for a partner may be formed naturally after several trials of cohabitation, or faster if mating occurs, so that the CS (e.g. neutral cues) and UCS (e.g. reward) occur in contingency and contiguity. Accordingly, treatment with a D2-type receptor agonists like QNP may accelerate the neural process (presumably in NAcc) in just three trials because it mimics the UCS.

Limbic and hypothalamic structures known previously to be involved in reward-related motivation in general (Berridge and Robinson, 1998), and sexual motivation in particular (Pfaus, 2009), are activated by conditioned odors paired with reward states. For example, Kippin et al. (2003) showed that males exposed to estrous odors responded with more Fos immunoreactivity (Fos-IR) in cells found in the accessory olfactory bulb, Nucleus accumbens (NAcc) shell and core, medial bed nucleus of the stria terminalis, medial amygdala, medial preoptic area, ventromedial hypothalamus, and ventral tegmental area. However, almond odor paired with sex induced more Fos-IR in the NAcc core, piriform cortex, anterior portion of the lateral hypothalamus, and basolateral amygdala. Those findings lead the authors to suggest that conditioned and pheromonal odors activate not only similar (i.e. NAcc core) but also independent pathways in the limbic system and hypothalamus that may be related to attention, reward, and reproductive outcomes. Other studies have also shown that hypothalamic and limbic neurons can respond to conditioned odors and consequently increase the levels of gonadotrophins, such as luteinizing hormone, and testosterone (Graham and Desjardins, 1980). Thus, an odor that gains sexual incentive value after conditioning may be capable of increasing sexual motivation via activation of these brain areas and neuroendocrine mechanisms.

#### 4.3. Non-contact erections

In a previous study we showed that males from the QNP group displayed more non-contact erections than control males following exposure to the scented male bearing the conditioned odor (compared to the number of erections evoked by exposure to a novel male partner) (Triana-Del Rio et al., 2011). More erections indicate a higher level of sexual arousal. In the present study we also assessed the frequency of non-contact erections in males exposed to a scented male partner and compared it with the frequency of erections during exposure to a sexually receptive female. As expected, males from the saline group displayed more non-contact erections in the presence of a sexually receptive female, and few in presence of another male rat.

However, males from the QNP group expressed the opposite pattern (Fig. 5). We have previously discussed that such results were not the pharmacological consequence of QNP alone. 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 our test occurred 4 days after the last injection of QNP, it is unlikely that the drug had an acute effect on those erections. Thus, we argue that males from the QNP group were more sexually aroused by the presence of the male, and less aroused by the receptive female. This suggests that our experimental protocol modified the target of sexual motivation, but not the capacity of the males to become sexually aroused.

Males from the QNP group also displayed female-like solicitations, a behavior normally observed when females are proceptive. Female solicitations are observed as a head-wise orientation to the male followed by a runaway, which forces the male to chase the female. Solicitations are thus a form of appetitive behavior that invites males to copulate, and have been used previously as a measure of female sexual desire for a partner (Pfaus et al., 2004). Males normally do not show this behavior, and in fact, only few control males did it at a very low frequency. We speculate that males from the QNP group expressed a rudimentary sexual desire for some kind of close interaction with the familiar stimulus male because they engaged in female-like solicitations toward the male and displayed NCEs in the presence of the male, behaviors not displayed by males in the saline group. However, males did not show hops, darts or lordosis, which normally occur during the bouts of preceptivity and receptivity in female rats. In addition, males did not mount the stimulus males or attempt any other form of copulation with them. Although it is tempting to speculate that the shift in preference away from the receptive female to the male represents a homosexual partner preference, it is obviously not a preference for copulation with the same sex partner, as the QNP males could have attempted to do so during the test but did not. Thus, we cautiously refer to this as a same-sex partner preference, with the nature of the preference being sociosexual. To our knowledge, male–male solicitations that follow the same pattern as female–male solicitations have not been reported previously in gonadally intact male rats. Further tests are necessary to determine the exact nature of this behavior.

## 5. Conclusion

The most accepted theory to explain homosexual partner preference is based on the organizational hypothesis (Phoenix et al., 1959), from which many other studies have assessed sexual dimorphism of the brain and 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). It is possible that neural structures organized during the perinatal period set the neural bases for unconditioned preferences. However, our results suggest that presumably unconditioned preferences can weaken (i.e. for a sexually receptive female), whereas conditioned preferences can be strengthened (i.e. for a scented male) following a process of conditioning with cohabitation under enhanced D2-type activity.

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