Pharmacology, Biochemistry and Behavior 180 (2019) 83–91

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

The neonatal treatment with clomipramine decreases sexual motivation and increases estrogen receptors expression in the septum of male rats: Effects of the apomorphine

Tania Molina-Jiménez[a], Martín Jiménez-Tlapa[a], Malinalli Brianza-Padilla[b], Rossana Citlali Zepeda[c], Marisela Hernández-González[d], Herlinda Bonilla-Jaime[e,*]

[a] Facultad de Química Farmacéutica Biológica, Universidad Veracruzana, Circuito Gonzalo Aguirre Beltrán Sn, Zona Universitaria, C.P. 91090 Xalapa-Enríquez, Veracruz, Mexico
[b] Departamento de Inmunología, Instituto Nacional de Cardiología Ignacio Chávez, Juan Badiano No.1, Col Sección XVI, Delegación Tlalpan, Zona de Hospitales, C.P. 01480, Ciudad de México, Mexico
[c] Centro de Investigaciones Biomédicas, Universidad Veracruzana, Dr. Luis Castelazo Ayala s/n, Col. Industrial Ánimas, 91190 Xalapa, Veracruz, Mexico
[d] Instituto de Neurociencias, Universidad de Guadalajara, Francisco de Quevedo 180, col. Arcos-Vallarta, CP 44130 Guadalajara, Jalisco, Mexico
[e] Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana-Iztapalapa, Apartado Postal 55 535, C.P. 09340 Ciudad de México, Mexico

ARTICLE INFO

Keywords:
Clomipramine
Dopaminergic system
Male sexual behavior
Estrogen receptors
Depression

ABSTRACT

Administering clomipramine during the early days of life induced several behavioral and neurochemical alterations in adult male rats, which resemble major depression disorder. The alterations included poor sexual performance, which is considered a reward-seeking behavior regulated by dopaminergic system. Given that estrogen receptors are expressed in different areas of the brain involved in regulating reproductive behavior, motivation and mood. The objective of this study was to analyze the effect of a non-selective dopamine agonist (apomorphine) on sexual incentive motivation in rats exposed to clomipramine (CMI) in the neonatal period. In addition, we evaluated the expression of mRNA ERα and ERβ in nucleus *accumbens* (NAcc) and septum of CMI rats. We found that only a few rats subjected to neonatal CMI treatment performed mounts, intromissions and ejaculations. Also, those rats spent less time exploring the sexual incentive zone and had lower preference scores; this effect was reverted by administering 0.1 mg/kg of apomorphine. Finally, the CMI rats presented higher levels of mRNA ERα and ERβ, only in septum area. These data indicate that neonatal treatment with CMI altered the expression of mRNA ERα and ERβ in the septum, which participates in regulating the motivational component of sexual behavior.

1. Introduction

Sexual motivation is which drives males to search for, and approach to receptive females in order to perform copulation (Agmo, 1999; Beach et al., 1956; Everitt, 1990). It requires the appropriate integration of neural, hormonal and sensorial processes that all together, allow perceive the sexual stimuli as incentives and potentially rewarding. Several structures of the brain reward circuitry are involved in sexual motivation, highlighting those that receive dopaminergic innervation from the ventral tegmental area (VTA); such as the medial preoptic area (mAPO), amygdale, prefrontal cortex, septum and nucleus *accumbens* (NAcc) (Ikemoto, 2010). Although the mAPO is the crucial structure for both, sexual motivation and execution (Paredes, 2003), other areas also exert an important modulation on copulatory behavior. For example, the lateral septum (LS) that receives input from dopaminergic cell groups in the VTA (Cornwall et al., 1990; Seifert et al., 1998), is involved not only in facilitating this behavior, but also in inhibiting heterotypical sexual behavior in male rats (Kondo et al., 1990). Similarly, the NAcc, considered as limbic-motor interface (Mogenson et al., 1980), participates in the processing of sexual stimuli and hence, the induction of sexual motivation (Floresco, 2015; Liu et al., 1998).

The functioning of these brain structures, like other areas implicated in the sexual behavior and mood, is regulated by hormones. In fact, several reports have shown that androgens and estrogens are necessary for performance of male sexual behavior as well as for expressing the motivational component (Attila et al., 2010; Hull and Dominguez, 2007). Concurrently, the two types of receptors at which are joined to estrogens, ERα and ERβ, are expressed in the septum, NAcc and mAPO

* Corresponding author.
E-mail address: bjh@xanum.uam.mx (H. Bonilla-Jaime).

https://doi.org/10.1016/j.pbb.2019.03.004
Received 2 November 2018; Received in revised form 20 February 2019; Accepted 19 March 2019
Available online 22 March 2019
0091-3057/

(Shughrue et al., 1998; Shughrue and Merchenthaler, 2001; Simerly et al., 1990; Wood and Newman, 1995).

On the other hand, dopamine (DA) is a neurotransmitter involved in mediating reward and motivation behaviors (Berridge, 2007; Hull et al., 1997; Paredes and Agmo, 2004). Some reports suggest that reducing dopaminergic transmission may induce deficit in sexual motivation of male rats (López and Ettenberg, 2001), while administering apomorphine (a non-selective DA agonist) increases the proportion of males that ejaculates and reverts sexual exhaustion, possibly as a result of the increase of sexual motivation (Mas et al., 1995; Rodríguez-Manzo, 1999). Similarly in hamsters, acute apomorphine treatment increases the number of ejaculations before the onset of long intromissions, a parameter that indicates attainment of sexual satiety (Arteaga et al., 2002). Some pharmacological data suggest that the regulation of the male sexual motivation in the NAcc is due to the presence of DA (Giuliano and Allard, 2001; Melis and Argiolas, 1995). In fact, it has been demonstrated that DA increases in the NAcc of male rats in anticipation of gaining access to a sexually-receptive female and during sexual behavior (Damsma et al., 1992; Pfaus and Phillips, 1991; Pleim et al., 1990), indicating that steroid hormones are not the only ones that regulate this behavior.

Nearly most of the works that has been studied sexual motivation during copulatory interactions of rats, measure parameters such as attention, persecution and mount latency, since are considered the most common indicator of male sexual motivation (Melis and Argiolas, 1995). Recently, the sexual incentive motivation test (SIM) has often been used, because it allows measure the sexual motivation independently of the copulatory interaction (Spiteri and Ågmo, 2006). This test evaluates approaching behaviors directed to a stimulus such as a receptive female (sexual incentive) or a conspecific male (social incentive). Thus, when rats spend more time exploring the sexual incentive zone their actions are interpreted as indicator of the intensity of sexual motivation, which has been correlated to the libido in humans (Agmo, 1999; Agmo et al., 2004; Agmo and Ellingsen, 2003; Spiteri and Ågmo, 2006; Vega Matuszcyk et al., 1998). Using this paradigm, Agmo et al. demonstrated that the administration of different doses of apomorphine (0.125, 0.25 and 0.5 mg/kg) had no effect on the sexual motivation of male rats, although subjects showed sluggish movements after the highest doses (Agmo, 2003a). Nevertheless, lower doses of apomorphine (5 μg/kg) increased copulatory behavior in ERα KO rats (Wersinger and Rissman, 2000), and the administration of 100 μg of apomorphine not only increased the frequency of mounts but also elicit penile erection, and increased grooming in rats subjected to stress (Niikura et al., 2002). In addition, doses of 25 and 50 μg of apomorphine decrease the intromission latency, indicating that lower doses increase copulatory parameters as well as sexual motivation (Guadarrama-Bazante et al., 2014). However, lower doses of apomorphine have not been evaluated using the SIM test. Likewise, the participation of the hormonal regulation on the sexual motivation has also investigated by mean the SIM test, showing that displaying sexual motivation requires the simultaneous stimulation of androgen and estrogen receptors (Attila et al., 2010).

In humans, the decrease of sexual motivation is one of the main problems associated to depression (Kennedy and Rizvi, 2009). In rats, early exposure to clomipramine (CMI, a tricyclic antidepressant that inhibits the reuptake of serotonin and noradrenaline) has been used as a neurodevelopmental model for studying depression (Willner and Mitchell, 2002) due to its ability to cause depressive-like symptoms during adulthood (Feng and Ma, 2002; Justel et al., 2011; Vijayakumar and Meti, 1999; Vogel et al., 1996, 1990). Furthermore, alterations in sexual performance were observed in male rats exposed to CMI during postnatal period, characterized by reduction in the number of mounts and intromissions preceding ejaculation, decreasing in the frequency of ejaculations and longer latencies of mounts, intromission and post-ejaculatory intervals (Bonilla-Jaime et al., 1998; Neill et al., 1990; Vogel et al., 1996). Besides, these rats did not only exhibit alteration in consummatory sexual behavior, but also in the motivational component evaluated in SIM test. Nevertheless, these alterations were reverted after the simultaneously administration of 17β-estradiol (E2) and 5-α-dihydrotestosterone (DHT) (Bonilla-Jaime et al., 2003; Limón-Morales et al., 2014a). Interestingly, no changes were observed in plasma DHT and E2 concentration in rat early exposed to CMI (Limón-Morales et al., 2014a), but it is possible that could disrupt normal estrogen expression in structures involved in sexual motivation.

In addition, the synthesis and release of neurotransmitters is influenced by E2 (Luine et al., 1997; Micevych et al., 1988), then changes in the levels of E2 are reflected in the levels of neurotransmitters. It has been also shown that administration of selective serotonin reuptake inhibitors (SSRI) inhibits the aromatase enzyme, resulting in reduced production of androgens and estrogens (Jacobsen et al., 2015). Thus, we hypothesized that dopaminergic disruption is involved in the alteration of sexual behavior. Therefore, one of the objectives of this study was to determined the effect of apomorphine on copulation and sexual incentive motivation of male rats treated neonatally with CMI. Moreover, with the aim to know if these adverse effects on sexual behavior are result of the altered estrogenic regulation on brain structures, we determined the effect of the neonatal CMI on the levels of mRNA ERα and ERβ in NAcc and septum of these rats.

2. Materials and methods

2.1. Subjects

Rats were obtained from the vivarium of the Universidad Autónoma Metropolitana, and all experiments were carried out in strict accordance with the Official Mexican Standards, NOM-062-ZOO-1999 (Agroalimentaria, n.d.) and the National Institute of Health Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). The litters were obtained from 12 pregnant Wistar rats. Three days after delivery, male pups were randomly cross-fostered to maintain a uniform number of pups in each litter (n = 6 pups/mother) (Bonilla-Jaime et al., 1998; Neill et al., 1990; Vázquez-Palacios et al., 2005; Vogel et al., 1988). The female pups were eliminated from this study. The animals were housed according to treatment (control or experimental), and all were kept on a 12 h light-dark cycle (lights on at 9:00, off at 21:00h) with *ad libitum* access to food and water. From neonatal days 8 to 21, the experimental group (CMI, n = 32) received clomipramine (15 mg/kg body weight in 0.1ml saline solution, s.c.), while the control pups (CTRL, n = 31) received vehicle only (0.1 ml of saline solution, s.c.). The dose of CMI was chosen due to its effectiveness in producing the behavioral and physiological abnormalities required by the study protocol (Bonilla-Jaime et al., 1998; Hartley et al., 1990; Neill et al., 1990; Vázquez-Palacios et al., 2005; Vogel et al., 1990). Pups were injected twice a day (9:00am and 18:00pm) while still with the mother. At 23 day of age, the pups were weaned, randomly housed in groups of 6, and maintained under standard conditions.

At the age of 3 months, both groups were subjected to three tests of male sexual behavior (MSB) to provide sexual experience. The groups were then exposed to a basal sexual incentive motivation test (SIM). Later, the animals from both groups were used in two experiments to: A) evaluate the effect of apomorphine administration on sexual motivation and, B) determine the levels of mRNA ERα and ERβ in the NAcc and septum.

2.2. Experimental design

2.2.1. Experiment 1. Effect of apomorphine administration on sexual motivation in CMI and CTRL male rats

This experiment was designed to stimulate and then revert the decrease of sexual motivation in rats exposed to neonatal treatment with

CMI. Animals from both groups, CTRL and CMI, were randomly subdivided as follows: vehicle (n = 6); 0.025mg/kg apomorphine (n = 6); 0.05 mg/kg apomorphine (n = 6); and 0.1 mg/kg apomorphine (n = 6). The rats were subjected to the SIM test 15 min after administration of apomorphine or saline i.p. (vehicle).

2.2.2. Experiment 2. Determination of the levels of mRNA ERα and ERβ in the NAcc and septum of rats

A second experiment was conducted to analyze the effect of neonatal treatment with CMI on the expression of ERα and ERβ receptors in the NAcc and septum. After 3 tests of male sexual behavior to give the rats sexual experience, animals from the CMI (n = 8) and CTRL (n = 7) groups distinct from those used in experiment 1, were sacrificed by decapitation. The NAcc and septum were then dissected to evaluate the expression of the ERα and β receptors by RT-PCR.

2.3. Drugs

Clomipramine hydrochloride (Sigma-Aldrich, St Louis, MO, USA) was dissolved in a freshly prepared 0.9% saline solution, and injected subcutaneously at a volume of 0.1 ml/weight. Apomorphine (Sigma-Aldrich, St Louis, MO, USA) was also dissolved in sterile 0.9% saline solution and injected intraperitoneal at doses of 0.025, 0.05 and 0.1mg/kg, 15 min before the SIM test.

2.4. Male sexual behavior test (MSB)

Males were tested for sexual behavior three times in order to induce sexual experience and corroborate alterations of the copulatory parameters caused by neonatal administration of CMI. For this test, each male rat was placed in a plexiglass arena (45 cm in diameter), allowing 5 min exploration, and after this time a receptive female rat was introduced as the stimulus. The female rats were made sexually receptive by administering estradiol benzoate (Sigma Chemical Co., St. Louis, MO, USA, at 10 μg/0.1 ml oil, SC) 48 h before the MDB test, and progesterone (Sigma Chemical Co., St. Louis, MO, USA, at 1 mg/0.1 ml oil, SC) 4 h before testing. The MSB test lasted 30min and was performed under dim red light 3h after the onset of the dark phase. The parameters recorded during the tests were: latency to first mount; latency to first intromission; latency to first ejaculation; number of mounts (mounts with pelvic thrusting); and number of intromissions (mounts with pelvic thrusting and penile insertion) during the first copulatory series. Also, the ejaculation frequency (number of ejaculations during 30 min of recording), and post-ejaculatory intervals (time between ejaculation and the subsequent intromission) were registered. The intromission ratio was calculated as follows: ratio between the number of intromissions and mounts plus the number of intromissions. The full description of male sexual behavior parameters has been detailed elsewhere (Agmo, 1997).

2.5. Sexual incentive motivation test (SIM)

One week before SIM testing, all experimental males were familiarized with the SIM arena during three 10-min sessions, separated by 48 h. During these sessions, the incentive animal cages were empty. The arena was carefully washed with a 5% ethanol solution between subjects during all sessions.

The experimental test consisted of two 10-min sessions (separated by 48h) in which each male rat was placed manually in the center of the arena under identical conditions to those of the familiarization assays, except that one of the incentive animal cages contained a sexually-receptive female (sexual incentive), and the other an intact male (social incentive). The position of the incentive animals was changed semi-randomly during the sessions to balance any possible position preference. During the SIM test, researchers measured the time that the male remained in the vicinity of each incentive cage and registered the time that the rat spent in each incentive zone, characterized by placing its two anterior paws inside the area. Motivational scores were calculated by the following formula: (time with female) / (time with female + time with male). The SIM test was performed in a rectangular arena (100 L × 50 W × 45 H cm). The floor and walls were made of black acrylic with one opening (25 × 25cm) on each long side. An incentive animal cage (25 L × 15 W × 25 H cm) was attached behind each opening. The front facing the arena was made of double wire mesh (separation = 1 cm; mesh size = 12 × 12 mm), so that the rats could hear and smell the animal in the cage but could not have direct physical contact. A virtual – "incentive" – zone that measured 29 × 21 cm was defined in front of each incentive animal cage. Detailed descriptions of this apparatus can be found elsewhere (Agmo, 2003b; Agmo et al., 2004).

2.6. RNA extraction and RT-PCR

Sexual experienced male rats (tested three times in MSB) of both treatments (CTRL and CMI) were euthanized by decapitation after the last evaluation of MSB test. The brains of each animal were removed and aseptically dissected at 4 °C. For this procedure, 0.3 g of tissue were obtained from the NAcc and septum and washed with saline, before pretreatment with 1 ml of RNA (QIAGEN Science, Germantown, MD, USA) for 24 h at room temperature. The tissues were stored at −70 °C until analysis. Once thawed, the excess RNA was removed by adding 1 ml of lysis reagent Quiazol to achieve homogenization. The extraction of total RNA from the tissues was performed by the phenol-chloroform method. The purity and integrity of the total RNA was analyzed with a Nano Drop-2000 analyzer (Thermo Fisher), absorbance was measured at 260 and 280nm, and the absorbance ratio was 1.90 ± 0.2. To confirm RNA integrity, 1 μg was run in 1% agarose gel.

Using the ImProm II reverse transcription system (Promega, Madison, WI, USA) two μg of total RNA was reverse-transcripted in the thermal cycler according to the provider's instructions (Applied Biosystems Gene Amp PCR System 2700HT, Foster City, USA), using the following cycle program: incubation for 5 min at 25 °C and 55 min extension at 42 °C. The enzyme was inactivated at 70 °C for 15 min and, finally, at 4 °C for 5 min. The cDNA obtained was amplified using SYBER Green Master Mix technology (Roche Molecular Biochemicals, Mannheim, Germany) containing 0.5nM of customized primers for ERα and ERβ (Table 1), fast star enzyme, PCR buffer, and 3.5mM $MgCl_2$, for a final volume of 10 μl. Reactions were evaluated in a LightCycler 2.0 real-time system (Roche Molecular Biochemicals) with 0.5 mM of primer (Table 1) under the following conditions: 40 cycles that included denaturalization at 95 °C for 10 s, alignment at 61 °C for 7 s, and amplification at 72 °C for 10 s. The identity and purity of the amplified products were verified by electrophoresis with 2% agarose gel with a molecular weight marker (100 bp DNA Ladder). The ΔCt values were calculated as follows: Ct gene of interest − Ct reference gene, with GAPDH as the reference gene.

2.7. Statistical analyses

The proportion of animals that displayed mounts, intromissions and ejaculations was analyzed by a Chi-square test, while the effect of

Table 1
Gene sequence used as forward (F) and reverse (R) primers for RT-PCR.

Gene	Sequence (F - sense/R - antisense)	Gene Bank	Product size (bp)
GAPDH	F - CCTGCACCACCAACTGC R - CAATGCCAGCCCCAGCA	NM_017008.4	453 bp
ER α	F - TTCACACCAAAGCCTCGGG R - TGCAGCAGCATCAGCGGA	NM_012689.1	337 bp
ER β	F - TCCCGGCAGCACCAGTAAC R - CCCAGATGCATAATCGCTGC	NM_012754.1	301 bp

neonatal treatment with CMI on copulatory parameters (latency to the first mount, intromission or ejaculation, number of mounts, number of intromissions, ejaculation frequency, post-ejaculatory intervals and the intromission ratio) and sexual motivation was evaluated using *t*-test. The effect of apomorphine treatment on sexual motivation (*i.e.*, time spent in the sexual incentive zone, time spent in the social incentive zone, and preference scores) was analyzed by two-way ANOVA using the factors neonatal treatment (CMI, CTRL) and treatment with apomorphine (Veh, 0.025, 0.05 and 0.1 mg/kg). When p values reached < 0.05, Student-Newman-Keuls *post hoc* test was used to determine the source of significance. For the analysis of the expression of mRNA ERα and ERβ in the NAcc and septum we used *t*-test to compare differences between the CMI and CTRL groups. Results are shown as mean ± standard error (SEM). All analyses were performed with the SigmaPlot 10 pack (San José, CA, USA).

3. Results

3.1. Effect of neonatal treatment with CMI on copulation and sexual incentive motivation (SIM)

The percentage of rats that displayed mounts, intromissions and ejaculations on the third MSB test was affected by treatment with CMI. Neonatal exposure to CMI reduced the percentage of rats that displayed mounts (18%, $x^2 = 13.88$, $p < 0.001$), intromissions (18%, $x^2 = 13.88$, $p = 0.001$) and ejaculations, compared to the animals treated with saline only (10%, $x^2 = 10.74$, $p = 0.001$).

The copulatory parameters are shown in Table 2. Rats subjected to a neonatal CMI showed an increase in the mount ($t\ (30) = -3.73$, $p = 0.001$) and intromission latencies ($t\ (28) = -6.17$, $p = 0.001$) compared to CTRL group. Moreover, only 2 of the 24 CMI rats reached ejaculation in the 30 min of the third sexual behavior test.

Fig. 1 shows the effect of neonatal treatment with saline and CMI on sexual incentive motivation in adult male rats. Motivation towards the sexual incentive diminished in the CMI rats [$t\ (46) = 3.38$, $p = 0.001$], but did not affect exploration in the social incentive zone [$t\ (46) = -0.63$, $p = 0.52$]. Also, the rats exposed to early administration of CMI had lower preference scores than those in the CTRL group ($t\ (46) = 2.38$, $p = 0.02$).

Table 2
Male sexual behavior parameters on the third behavioral test.

Copulatory behavior	CTRL	CMI
LM	59.87 ± 17.22 n = 24	492.75 ± 200.62* n = 8
LI	80.20 ± 18.29 n = 24	796.33 ± 232.43* n = 6
LE	809.31 ± 102.69 n = 22	854.50 ± 144.50 n = 2
PR	330.61 ± 28.24 n = 18	417 ± 0 n = 1
NM	11.00 ± 1.77 n = 22	19.00 ± 10.00 n = 2
NI	12.31 ± 1.03 n = 22	14.00 ± 1.00 n = 2
FE	2.00 ± 0.19 n = 22	1.50 ± 0.50 n = 2
IR	0.56 ± 0.04 n = 22	0.46 ± 0.12 n = 2

Neonatal CMI rats had longer latencies to perform mounts and intromissions compared to control group. Data were presented as mean ± SEM;. Data were presented as mean ± SEM. Abbreviations: EF = ejaculation frequency; ML = mount latency; IL = intromission latency; EL = ejaculation latency; NM = number of mounts; NI = number of intromissions; IR = intromission ratio; PR = refractory period.

* *t*-Test, $p < 0.05$ compared to the CTRL group.

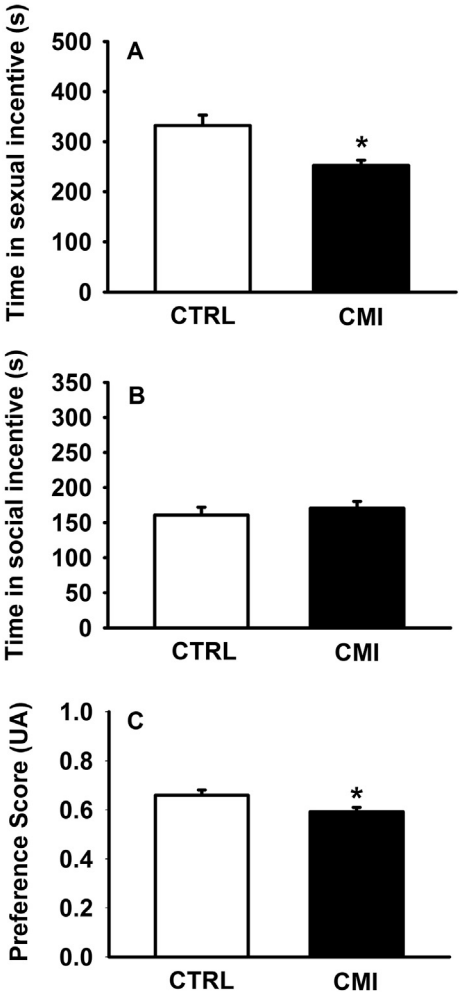

Fig. 1. Effect of neonatal treatment with CMI on the SIM test in adult male rats. CMI treatment early in life reduces the time spent in the sexual incentive area and preference scores. *t*-Test, *p < 0.05 compared to the CTRL group. Data presented as mean ± SEM. Abbreviations: CTRL: control; CMI: clomipramine.

3.2. Effect of apomorphine on sexual motivation in CMI and CTRL male rats

Fig. 2 presents the effect of the different doses of apomorphine on the SIM test in both groups (CTRL and CMI). The two-way ANOVA revealed that there were not statistical differences in the time spent in the sexual incentive area [$F_{(1,40)} = 0.99$, $p = 0.32$, NS]. However, results do show differences among the different apomorphine treatments [$F_{(3,40)} = 4.61$, $p = 0.007$]. The *post-hoc test* indicated that, regardless of group, the 0.1 mg/kg dose of apomorphine increased the time spent in the sexual incentive zone, though the interaction group × apomorphine treatment was not significant [$F_{(3,40)} = 0.84$, $p = 0.47$, NS]. The analysis of time spent in the social incentive area indicated differences between the CMI and CTRL groups [$F_{(1,40)} = 17.32$, $p = 0.001$], as the former spent more time exploring that zone. Regardless of group, the 0.05 mg/kg dose of apomorphine increased the time spent exploring the social incentive zone [$F_{(3,40)} = 3.41$, $p = 0.026$], compared to the vehicle and the 0.025 and 0.1 mg/kg doses. The interaction group × apomorphine treatment was not significant [$F_{(3,40)} = 1.27$, $p = 0.29$, NS], but the analysis of preference scores indicated between-group differences [$F_{(1,40)} = 8.13$, $p = 0.007$], as the CMI subjects had lower preference scores than CTRL group. We also found that, regardless of group, the 0.1 mg/kg dose of apomorphine increased preference scores [$F_{(3,40)} = 3.20$, $p = 0.033$, NS] compared to the vehicle and the

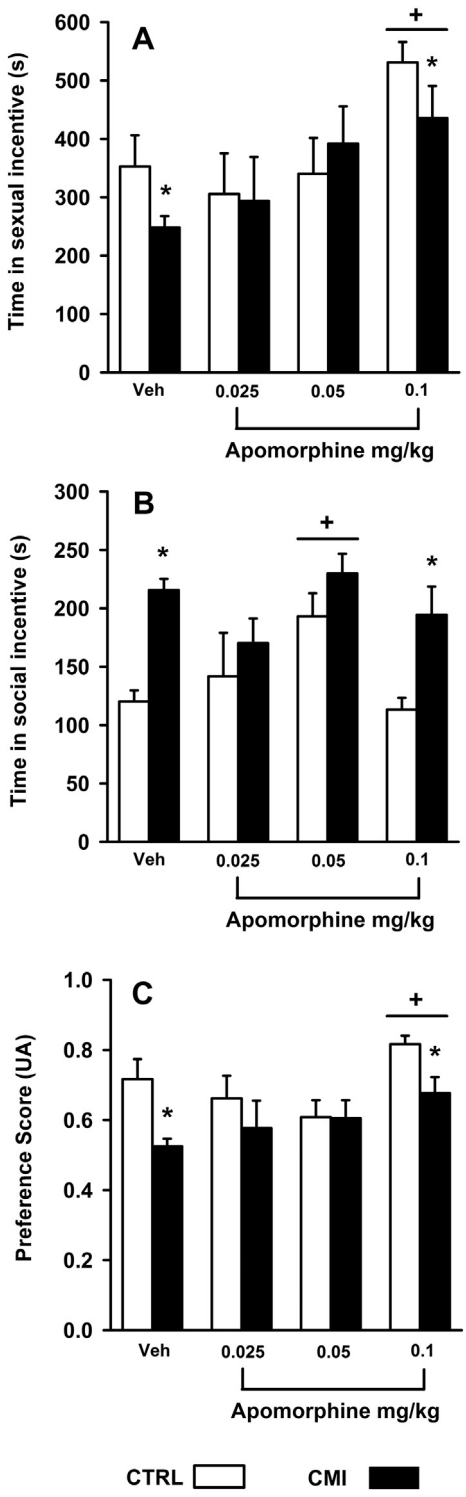

Fig. 2. Effect of apomorphine on the SIM test in the CMI and CTRL rats. CMI treatment early in life reduces the time spent in the sexual incentive area (A) and in preference scores (C) at the dose of 0.1 mg/kg of apomorphine. Also, the increase in the time spent in the social zone was observed at the 0.05 mg/kg dose of apomorphine (B). Student-Newman-Keuls *post hoc*, *p < 0.05 compared to the CTRL group; $^{+}$p < 0.05 compared to Veh. Data presented as mean ± SEM. Abbreviations: CTRL: control; CMI: clomipramine.

0.025 and 0.05 mg/kg doses. Interaction between factors was not significant [$F_{(3,40)} = 1.19$, p = 0.32, NS].

3.3. Effect of neonatal clomipramine administration on the expression of ERα and ERβ in the NAcc and septum

Fig. 3 shows the results of ERα and ERβ mRNA levels in the septum and NAcc. In this case, early exposure to CMI induced a significant increase in the expression of the mRNA of ERα [t (13) = −3531, p = 0,004] and ERβ [t (13) = −4508, p = 0,001] in the septum compared to CTRL group (panels a and b), though no modification in the expression of the mRNA of ERα [t (11) = −0.08, p = 0.931] and ERβ [t (15) = −1.142, p = 0.271] was found in the NAcc, (panels c and d).

4. Discussion

The present study corroborates earlier findings that postnatal exposure to CMI decreases sexual behavior, as only a few of the rats exposed to CMI displayed mounts, intromissions and ejaculations. Furthermore, those rats had larger latencies to display mounting and intromission behaviors and spent less time exploring the sexual incentive zone. Finally, their preference scores were lower. These results are similar to those reported by other authors (Bonilla-Jaime et al., 1998; Limón-Morales et al., 2014a; Neill et al., 1990; Vogel et al., 1990). In contrast, neonatal administration of CMI did not affect the time spent in the social incentive zone, a result that complements findings from studies which reported that prenatal treatment with CMI or Lu 10-134-C (SSRI) had no effect on social interaction (File and Tucker, 1983; Hansen et al., 1997). It is important to mention that the serotonergic system is one of the earliest monoaminergic systems to appear during early brain development, and the one that plays an important role in such neuronal processes as proliferation, migration, differentiation and synaptogenesis (Glover and Clinton, 2016; Hornung, 2003). Hence, pharmacological manipulation of this system during gestation and postnatal stages can potentially cause disruptions in brain structure, neuronal circuits and behaviors including sexual performance. Thereby, the present study corroborates that neonatal administration of CMI causes a deterioration of the executory and motivational components of male sexual behavior, but does not affect such social aspects as interaction with other male conspecifics.

Sexual behavior in rodents involves consummatory aspects that include a series of movements (mounts, intromission and ejaculations) that complete copulation. However, prior to consummation, the motivational component of sexual behavior functions as an important guide that leads the male to approach a receptive female in order to initiate the display of the sexual repertory (Fabre-Nys et al., 2003; Hull and Dominguez, 2007; Paredes and Vazquez, 1999). Motivation is regulated by the mesocorticolimbic dopamine circuit, which gives a motivational valence to stimuli with a biological meaning, including sexual behavior (Everitt, 1990; Pfaus, 2009; Yoest et al., 2014). Therefore, sexual motivation involves all approaching behaviors towards a sexually receptive female (Agmo, 2011, 2003b). Moreover, it has been reported that the odor of a receptive female increases extracellular DA in the NAcc (Fujiwara and Chiba, 2018). Also, an increased in extracellular DA in the mPOA was observed during precopulatory period as well as copulatory displaying of animals (Hull et al., 1995). Thus, these circuits are important for the association of sexual reward through the displaying of a behavioral repertory such as copulation (Fujiwara and Chiba, 2018; Hull and Dominguez, 2007).

In this study, three different doses of apomorphine (0.25, 0.05 and 0.1 mg/kg) were administered to both groups of rats (CTRL and CMI). Results show that the 0.1 mg/kg dose increased the time spent in the sexual incentive zone and preference scores in the CMI group, compared to the animals treated only with vehicle. Other studies reported that higher doses (0.125, 0.25 and 0.5mg/kg) of apomorphine did not

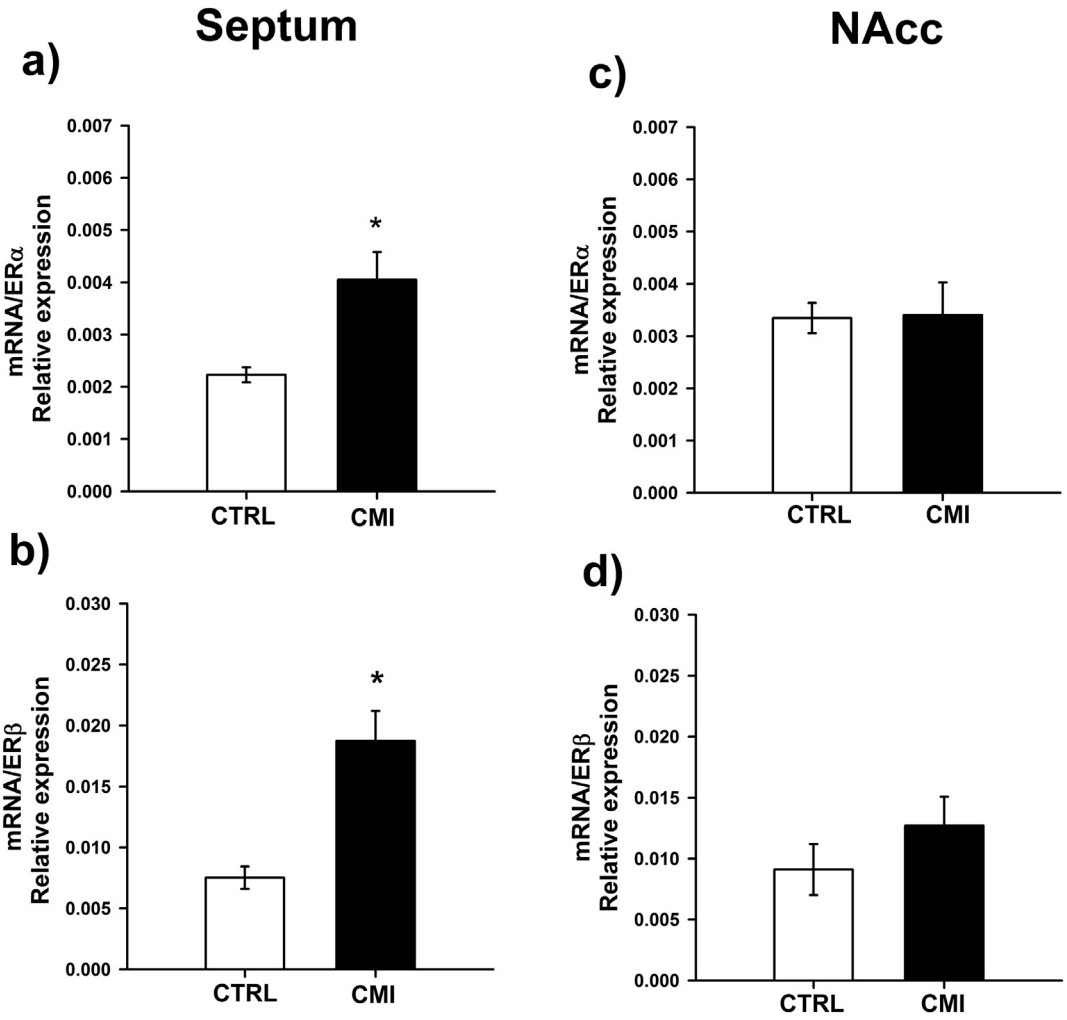

Fig. 3. Levels of ERα and ERβ mRNA in the septum and NAcc of CMI rats. The CMI rats had increased expression of ERα and ERβ mRNA levels, but only in the septum (a, b). No differences in the expression of mRNA of ERα and ERβ were detected in the NAcc (c, d) *t*-test, *p < 0.05 compared to the CTRL group. Data presented as mean ± SEM. Abbreviations: CTRL: control; CMI: clomipramine.

affect sexual motivation and the rats so treated presented alterations in their ambulatory activity (Agmo, 2003a). However, some others had shown that intraperitoneal or subcutaneous administration of apomorphine (100 μg in 0.1 cc or 5 μg/kg) not only activates copulatory behavior but also elicits penile erections, increased genital grooming, and restored copulatory behavior in stressed rats (Niikura et al., 2002; Wersinger and Rissman, 2000). Consistent with this, lower doses of apomorphine (25 and 100 μm/kg) reduced intromission and ejaculation latencies in both sexually experienced rats and young rats (Guadarrama-Bazante et al., 2014). Moreover, Paglietti et al. (1978) reported that doses of apomorphine of 25, 50 and 100 μg/kg, reduced intromission latencies and the post ejaculatory interval in sexually experienced male rats (Paglietti et al., 1978). Our study, meanwhile, found that a lower dose (0.1 mg/kg) of apomorphine was effective in improving sexual motivation in both groups (CMI and CTRL), with a greater effect on the control group. Thus, these data indicate that apomorphine facilitates the copulatory and motivational components of sexual behavior that were altered as a result of the postnatal treatment with CMI.

Likewise, E2 is important for displaying sexual behavior and motivation; ER are well-distributed in the central nervous system (CNS), specifically in the NAcc and septum, two structures involved in motivational and emotional processes (Salgado and Kaplitt, 2015; Sheehan et al., 2004). We evaluated whether exposure to treatment with clomipramine in the postnatal period modifies the expression of ERα and ERβ receptors. Results showed increase in the expression of mRNA of ERα and ERβ in the septum of CMI rats, with no observable changes of expression in the NAcc. Even so, Chavez et al. (2010) analyzed the effect of E2 on dopaminergic and serotonergic regulation by measuring its effects on the density of dopamine transporters (DAT), dopamine D_1 and D_2 receptors, serotonin transporters (SERT), 5-HT_{1A} and 5-HT_{2A} receptors using radioligand binding autoradiography. Results showed that E2 treatment completely reversed the low DAT and increased D_2 receptor density in NAcc induced by OVX rats to a lower level than those observed in intact rats, without effecting the serotonergic system (Chavez et al., 2010). In addition, E2 modulates DAT activity through a phosphorylation-dependent alteration of dopamine D_2/G protein coupling (Thompson and Certain, 2005). We did not found modifications on the expression of the mRNA of ERα and ERβ in NAcc by neonatal administration of CMI, which could indicate that even when the NAcc is a structure involved in motivational process, the early treatment with CMI did not affect the expression of both ER. On the other hand, lateral septum contributes to female sexual behavior in an inhibitory manner (Yamanouchi, 1997), while in males, the lesion of LS and dorsal raphe nucleus increases the behavior of lordosis (Kakeyama and Yamanouchi, 1993; Nance et al., 1975), suggesting that E2 releases inhibition on LS, unlike males, that inhibition in male LS may not be released by the direct action of estrogen (Satou and Yamanouchi, 1999).

In this case, CMI is a serotonin reuptake inhibitor and in a minor way a noradrenaline reuptake inhibitor, and alterations in the

serotonergic and noradrenergic systems have been reported in frontal cortex, hippocampus, raphe nucleus, septum and hypothalamus of rats exposed to CMI in neonatal period (Limón-Morales et al., 2014b; Vijayakumar and Meti, 1999). Similarly, the early administration of CMI affects the density of ER only in the septum, altering the inhibitory effect of this structure, by both, altering the adequate processing of the stimuli emitted by the receptive female in the incentive sexual motivation test so that they presented less time in the incentive zone and lower preference. The NAcc participates in the adequate processing and allocation of incentive value of the stimuli emitted by non-accessible females and it has been shown that DA levels increases in this structure in the presence of only receptive females (Liu et al., 1998); and that the presence of additional proximal stimulation during copulation may overcome the deficit induced by DA depletions or lesion in NAcc (Liu et al., 1998), effect that was observed with the administration of apomorphine in the CMI rats. Therefore, it is possible that the alterations in sexual motivation in CMI rats were induced by the neonatal stage manipulation of serotonergic system that modifies the expression of both α and β ER, in the septum, although some studies in fish and rodents show that fluoxetine (FLX), a SSRI, disrupts reproductive functions by reducing the expression of ER in the hypothalamus and also can act as an agonist (Mennigen et al., 2017, 2008; Müller et al., 2012).

Furthermore, the administration of CMI in postnatal days is also considered as a development animal model for studying depression. In this aspect, some studies have reported that depression may be accompanied by an alteration in the expression of ER in different brain areas (Ostlund et al., 2003; Perlman et al., 2004); *e.g.*, the increase of mRNA ERα in the hippocampus and dorsolateral prefrontal cortex in depressed patients, with a decrease in the mRNA of ERα in the basolateral amygdala (Perlman et al., 2005, 2004). Moreover, animal models of depression have shown a decrease in the expression of ERα and ERβ in the amygdala, hippocampus and prefrontal cortex in rodents subjected to chronic but unpredictable mild stress (Sharma and Thakur, 2015), while lower ERα expression levels in the amygdala were detected in Flinders Sensitive Line rats (Osterlund et al., 1999). In our results we observed an increased in mRNA of ERα and ERβ in septum, a structure involved in regulation of emotional stated and motivation (Sheehan et al., 2004). Then, all these data indicate that alterations of ER mRNA levels occur during depressive states in different brain, including septum.

In addition, a previous study showed that neonatal treatment with CMI did not affect plasma DHT and E2 concentrations, but decreased the copulatory parameters and the motivational component of sexual behavior, though these alterations were reverted by peripheral administration of E2 plus DHT (Limón-Morales et al., 2014a). At this time, there is no evidence of effects on cerebral DHT and E2 concentrations in rats with long-term alterations of sexual behavior caused by neonatal administration of CMI. It may be that brain testosterone and estradiol concentrations in these rats were affected by the early pharmacological manipulation of the serotonergic system, and that the absence of this hormone generated up-regulation of ER expression in the septum. This suggests that the serotonergic system is important for the adequate development of reproductive circuits in the brain, including ER. Consistent with this, the destruction of serotonergic neurons in the dorsal raphe increased the expression of ERα in the hypothalamus, indicating that the serotonergic neurons in the dorsal raphe nucleus exert an inhibitory function on ERα expression (Ito et al., 2014). Also, administration of SSRI inhibits the aromatase enzyme, resulting in reduced production of androgens and estrogens (Jacobsen et al., 2015). However, no information exists about the effect of early exposure to antidepressants on ER expression in the cerebral structures involved in regulating sexual behavior. In fact, our results show – for the first time – that neonatal treatment with CMI alters the expression of ERα and ERβ in the septum.

In conclusion, neonatal treatment with CMI altered consummatory behavior and reduced incentive sexual motivation. However, administration of low doses of apomorphine restored sexual motivation. Also, rats exposed to neonatal CMI treatment displayed up regulation of ERα and ERβ in the septum, perhaps due to lower cerebral estradiol levels. These data suggest that decreased sexual motivation and execution of male rats treated with CMI in the neonatal period, could be result of an altered dopaminergic regulation, or the increased expression of mRNA ERα and ERβ in the septum, which probably reduced the capacity of the adult male rats to adequately process the sexual stimuli emitted for the receptive female in the SIM test. Nevertheless, the evaluation of ER in other brain structures involved in the regulation of male sexual behavior could be one of the limitations of the study.

Acknowledgements

During this research, TMJ (CVU: 209784) received a scholarship from the Estancia Posdoctoral Vinculada al Fortalecimiento de la Calidad del Posgrado Nacional program (COVIA-0890-2016) granted by Mexico's Consejo Nacional de Ciencia y Tecnología (CONACyT). We thank the Laboratorio de Biología Molecular de Ciencias Biológicas y de la Salud (UAM-I) for authorizing the use of the specialized equipment required for this study.

References

Agmo, A., 1997. Male rat sexual behavior. Brain Res. Protocol. 1, 203–209. https://doi.org/10.1016/S1385-299X(96)00036-0.

Agmo, A., 1999. Sexual motivation–an inquiry into events determining the occurrence of sexual behavior. Behav. Brain Res. 105, 129–150. https://doi.org/10.1016/S0166-4328(99)00088-1.

Agmo, A., 2003a. Lack of opioid or dopaminergic effects on unconditioned sexual incentive motivation in male rats. Behav. Neurosci. 117, 55–68. https://doi.org/10.1037/0735-7036.117.1.3.

Agmo, A., 2003b. Unconditioned sexual incentive motivation in the male Norway rat (Rattus norvegicus). J. Comp. Psychol. Wash. DC 1983 117, 3–14. https://doi.org/10.1037/0735-7036.117.1.3.

Agmo, A., 2011. On the intricate relationship between sexual motivation and arousal. Horm. Behav. 59, 681–688. https://doi.org/10.1016/j.yhbeh.2010.08.013.

Agmo, A., Ellingsen, E., 2003. Relevance of non-human animal studies to the understanding of human sexuality. Scand. J. Psychol. 44, 293–301. https://doi.org/10.1111/1467-9450.00348.

Agmo, A., Turi, A.L., Ellingsen, E., Kaspersen, H., 2004. Preclinical models of sexual desire: conceptual and behavioral analyses. Pharmacol. Biochem. Behav. 78, 379–404. https://doi.org/10.1016/j.pbb.2004.04.013.

Agroalimentaria, S.N. de S., Inocuidad y Calidad, n.d. NOM-062-ZOO-1999 [WWW Document]. gob.mx. URL http://www.gob.mx/senasica/documentos/nom-062-zoo-1999 (accessed 9.13.18).

Arteaga, M., Motte-Lara, J., Velázquez-Moctezuma, J., 2002. Effects of yohimbine and apomorphine on the male sexual behaviour pattern of the golden hamster (Mesocricetus auratus). Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol. 12, 39–45. https://doi.org/10.1016/S0924-977X(01)00134-1.

Attila, M., Oksala, R., Agmo, A., 2010. Sexual incentive motivation in male rats requires both androgens and estrogens. Horm. Behav. 58, 341–351. https://doi.org/10.1016/j.yhbeh.2009.08.011.

Beach, F.A., Conovitz, M.W., Goldstein, A.C., Steinberg, F., 1956. Experimental inhibition and restoration of mating behavior in male rats. J. Genet. Psychol. 89, 165–181. https://doi.org/10.1080/00221325.1956.10534212.

Berridge, K.C., 2007. The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology 191, 391–431. https://doi.org/10.1007/s00213-006-0578-x.

Bonilla-Jaime, H., Retana-Marquez, S., Velazquez-Moctezuma, J., 1998. Pharmacological features of masculine sexual behavior in an animal model of depression. Pharmacol. Biochem. Behav. 60, 39–45. https://doi.org/10.1016/S0091-3057(97)00484-X.

Bonilla-Jaime, H., Retana-Marquez, S., Vazquez-Palacios, G., Velázquez-Moctezuma, J., 2003. Corticosterone and testosterone levels after chronic stress in an animal model of depression. Neuropsychobiology 48, 55–58. https://doi.org/10.1159/000072877.

Chavez, C., Hollaus, M., Scarr, E., Pavey, G., Gogos, A., van den Buuse, M., 2010. The effect of estrogen on dopamine and serotonin receptor and transporter levels in the brain: an autoradiography study. Brain Res. 1321, 51–59. https://doi.org/10.1016/j.brainres.2009.12.093.

Cornwall, J., Cooper, J.D., Phillipson, O.T., 1990. Afferent and efferent connections of the laterodorsal tegmental nucleus in the rat. Brain Res. Bull. 25, 271–284. https://doi.org/10.1016/0361-9230(90)90072-8.

Damsma, G., Pfaus, J.G., Wenkstern, D., Phillips, A.G., Fibiger, H.C., 1992. Sexual behavior increases dopamine transmission in the nucleus accumbens and striatum of male rats: comparison with novelty and locomotion. Behav. Neurosci. 106, 181–191. https://doi.org/10.1037/0735-7044.106.1.181.

Everitt, B.J., 1990. Sexual motivation: a neural and behavioural analysis of the mechanisms underlying appetitive and copulatory responses of male rats. Neurosci. Biobehav. Rev. 14, 217–232. https://doi.org/10.1016/S0149-7634(05)80222-2.
Fabre-Nys, C., Chesneau, D., de la Riva, C., Hinton, M.R., Locatelli, A., Ohkura, S., Kendrick, K.M., 2003. Biphasic role of dopamine on female sexual behaviour via D2 receptors in the mediobasal hypothalamus. Neuropharmacology 44, 354–366. https://doi.org/10.1016/S0028-3908(02)00410-0.
Feng, P., Ma, Y., 2002. Clomipramine suppresses postnatal REM sleep without increasing wakefulness: implications for the production of depressive behaviors. Sleep 25, 177–184. https://doi.org/10.1093/sleep/25.2.177.
File, S.E., Tucker, J.C., 1983. Neonatal clomipramine treatment in the rat does not affect social, sexual and exploratory behaviors in adulthood. Neurobehav. Toxicol. Teratol. 5, 3–8.
Floresco, S.B., 2015. The nucleus accumbens: an interface between cognition, emotion, and action. Annu. Rev. Psychol. 66, 25–52. https://doi.org/10.1146/annurev-psych-010213-115159.
Fujiwara, M., Chiba, A., 2018. Sexual odor preference and dopamine release in the nucleus accumbens by estrous olfactory cues in sexually naïve and experienced male rats. Physiol. Behav. 185, 95–102. https://doi.org/10.1016/j.physbeh.2017.12.029.
Giuliano, F., Allard, J., 2001. Dopamine and sexual function. Int. J. Impot. Res. 13, S18. https://doi.org/10.1038/sj.ijir.3900719.
Glover, M.E., Clinton, S.M., 2016. Of rodents and humans: a comparative review of the neurobehavioral effects of early life SSRI exposure in preclinical and clinical research. Int. J. Dev. Neurosci. Off. J. Int. Soc. Dev. Neurosci. 51, 50–72. https://doi.org/10.1016/j.ijdevneu.2016.04.008.
Guadarrama-Bazante, I.L., Canseco-Alba, A., Rodríguez-Manzo, G., 2014. Dopamine receptors play distinct roles in sexual behavior expression of rats with a different sexual motivational tone. Behav. Pharmacol. 25, 684–694. https://doi.org/10.1097/FBP.0000000000000086.
Hansen, H.H., Sánchez, C., Meier, E., 1997. Neonatal administration of the selective serotonin reuptake inhibitor Lu 10-134-C increases forced swimming-induced immobility in adult rats: a putative animal model of depression? J. Pharmacol. Exp. Ther. 283, 1333–1341.
Hartley, P., Neill, D., Hagler, M., Kors, D., Vogel, G., 1990. Procedure- and age-dependent hyperactivity in a new animal model of endogenous depression. Neurosci. Biobehav. Rev. 14, 69–72. https://doi.org/10.1016/S0149-7634(05)80161-7.
Hornung, J.-P., 2003. The human raphe nuclei and the serotonergic system. J. Chem. Neuroanat. 26, 331–343. https://doi.org/10.1016/j.jchemneu.2003.10.002.
Hull, E.M., Dominguez, J.M., 2007. Sexual behavior in male rodents. Horm. Behav. 52, 45–55. https://doi.org/10.1016/j.yhbeh.2007.03.030.
Hull, E.M., Du, J., Lorrain, D.S., Matuszewich, L., 1995. Extracellular dopamine in the medial preoptic area: implications for sexual motivation and hormonal control of copulation. J. Neurosci. 15, 7465–7471. https://doi.org/10.1523/JNEUROSCI.
Hull, E.M., Du, J., Lorrain, D.S., Matuszewich, L., 1997. Testosterone, preoptic dopamine, and copulation in male rats. Brain Res. Bull. 44, 327–333. https://doi.org/10.1016/S0361-9230(97)00211-6.
Ikemoto, S., 2010. Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory. Neurosci. Biobehav. Rev. 35, 129–150. https://doi.org/10.1016/j.neubiorev.2010.02.001.
Ito, H., Shimogawa, Y., Kohagura, D., Moriizumi, T., Yamanouchi, K., 2014. Inhibitory role of the serotonergic system on estrogen receptor α expression in the female rat hypothalamus. Neurosci. Lett. 583, 194–198. https://doi.org/10.1016/j.neulet.2014.09.043.
Jacobsen, N.W., Hansen, C.H., Nellemann, C., Styrishave, B., Halling-Sørensen, B., 2015. Effects of selective serotonin reuptake inhibitors on three sex steroids in two versions of the aromatase enzyme inhibition assay and in the H295R cell assay. Toxicol. Vitro Int. J. Publ. Assoc. BIBRA 29, 1729–1735. https://doi.org/10.1016/j.tiv.2015.07.005.
Justel, N., Bentosela, M., Mustaca, A., Ruetti, E., 2011. Neonatal treatment with clomipramine and depression: a review of behavioral and physiological findings. Interdisciplinaria 28, 207–220.
Kakeyama, M., Yamanouchi, K., 1993. Female sexual behaviors in male rats with dorsal raphe nucleus lesions: treatment with p-chlorophenylalanine. Brain Res. Bull. 30, 705–709. https://doi.org/10.1016/0361-9230(93)90104-J.
Kennedy, S.H., Rizvi, S., 2009. Sexual dysfunction, depression, and the impact of antidepressants. J. Clin. Psychopharmacol. 29, 157–164. https://doi.org/10.1097/JCP.0b013e31819c76e9.
Kondo, Y., Shinoda, A., Yamanouchi, K., Arai, Y., 1990. Role of septum and preoptic area in regulating masculine and feminine sexual behavior in male rats. Horm. Behav. 24, 421–434. https://doi.org/10.1016/0018-506X(90)90019-T.
Limón-Morales, O., Soria-Fregozo, C., Arteaga-Silva, M., González, M.H., Vázquez-Palacios, G., Bonilla-Jaime, H., 2014a. Hormone replacement with 17β-estradiol plus dihydrotestosterone restores male sexual behavior in rats treated neonatally with clomipramine. Horm. Behav. 66, 820–827. https://doi.org/10.1016/j.yhbeh.2014.09.014.
Limón-Morales, O., Soria-Fregozo, C., Arteaga-Silva, M., Vázquez-Palacios, G., Bonilla-Jaime, H., 2014b. Altered expression of 5-HT1A receptors in adult rats induced by neonatal treatment with clomipramine. Physiol. Behav. 124, 37–44. https://doi.org/10.1016/j.physbeh.2013.10.026.
Liu, Y.-C., Sachs, B.D., Salamone, J.D., 1998. Sexual behavior in male rats after radiofrequency or dopamine-depleting lesions in nucleus accumbens. Pharmacol. Biochem. Behav. 60, 585–592. https://doi.org/10.1016/S0091-3057(98)00022-7.
López, H.H., Ettenberg, A., 2001. Dopamine antagonism attenuates the unconditioned incentive value of estrous female cues. Pharmacol. Biochem. Behav. 68, 411–416. https://doi.org/10.1016/S0091-3057(00)00472-X.
Luine, V.N., Grattan, D.R., Selmanoff, M., 1997. Gonadal hormones alter hypothalamic GABA and glutamate levels. Brain Res. 747, 165–168. https://doi.org/10.1016/S0006-8993(96)01255-3.
Mas, M., Fumero, B., Perez-Rodriguez, I., 1995. Induction of mating behavior by apomorphine in sexually sated rats. Eur. J. Pharmacol. 280, 331–334. https://doi.org/10.1016/0014-2999(95)00270-U.
Melis, M.R., Argiolas, A., 1995. Dopamine and sexual behavior. Neurosci. Biobehav. Rev. 19, 19–38. https://doi.org/10.1016/0149-7634(94)00020-2.
Mennigen, J.A., Martyniuk, C.J., Crump, K., Xiong, H., Zhao, E., Popesku, J., Anisman, H., Cossins, A.R., Xia, X., Trudeau, V.L., 2008. Effects of fluoxetine on the reproductive axis of female goldfish (Carassius auratus). Physiol. Genomics 35, 273–282. https://doi.org/10.1152/physiolgenomics.90263.2008.
Mennigen, J.A., Zamora, J.M., Chang, J.P., Trudeau, V.L., 2017. Endocrine disrupting effects of waterborne fluoxetine exposure on the reproductive axis of female goldfish, Carassius auratus. Comp. Biochem. Physiol. Toxicol. Pharmacol. CBP 202, 70–78. https://doi.org/10.1016/j.cbpc.2017.08.003.
Micevych, P.E., Matt, D.W., Go, V.L.W., 1988. Concentrations of cholecystokinin, substance P, and bombesin in discrete regions of male and female rat brain: sex differences and estrogen effects. Exp. Neurol. 100, 416–425. https://doi.org/10.1016/0014-4886(88)90119-7.
Mogenson, G.J., Jones, D.L., Yim, C.Y., 1980. From motivation to action: functional interface between the limbic system and the motor system. Prog. Neurobiol. 14, 69–97. https://doi.org/10.1016/0301-0082(80)90018-0.
Müller, J.C., Imazaki, P.H., Boareto, A.C., Lourenço, E.L.B., Golin, M., Vechi, M.F., Lombardi, N.F., Minatovicz, B.C., Scippo, M.-L., Martino-Andrade, A.J., Dalsenter, P.R., 2012. In vivo and in vitro estrogenic activity of the antidepressant fluoxetine. Reprod. Toxicol. 34, 80–85. https://doi.org/10.1016/j.reprotox.2012.04.001.
Nance, D.M., Shryne, J., Gorski, R.A., 1975. Facilitation of female sexual behavior in male rats by septal lesions: an interaction with estrogen. Horm. Behav. 6, 289–299. https://doi.org/10.1016/0018-506X(75)90015-X.
National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011. Guide for the care and use of laboratory animals. In: The National Academies Collection: Reports Funded by National Institutes of Health, 8th ed. National Academies Press (US), Washington (DC).
Neill, D., Vogel, G., Hagler, M., Kors, D., Hennessey, A., 1990. Diminished sexual activity in a new animal model of endogenous depression. Neurosci. Biobehav. Rev. 14, 73–76. https://doi.org/10.1016/S0149-7634(05)80162-9.
Niikura, S., Yokoyama, O., Komatsu, K., Yotsuyanagi, S., Mizuno, T., Namiki, M., 2002. A causative factor of copulatory disorder in rats following social stress. J. Urol. 168, 843–849. https://doi.org/10.1016/S0022-5347(05)64754-4.
Osterlund, M.K., Overstreet, D.H., Hurd, Y.L., 1999. The flinders sensitive line rats, a genetic model of depression, show abnormal serotonin receptor mRNA expression in the brain that is reversed by 17beta-estradiol. Brain Res. Mol. Brain Res. 74, 158–166. https://doi.org/10.1016/S0169-328X(99)00274-0.
Ostlund, H., Keller, E., Hurd, Y.L., 2003. Estrogen receptor gene expression in relation to neuropsychiatric disorders. Ann. N. Y. Acad. Sci. 1007, 54–63. https://doi.org/10.1016/S0301-0082(00)00059-9.
Paglietti, E., Quarantotti, B.P., Mereu, G., Gessa, G.L., 1978. Apomorphine and L-DOPA lower ejaculation threshold in the male rat. Physiol. Behav. 20, 559–562. https://doi.org/10.1016/0031-9384(78)90247-0.
Paredes, R.G., 2003. Medial preoptic area/anterior hypothalamus and sexual motivation. Scand. J. Psychol. 44, 203–212. https://doi.org/10.1111/1467-9450.00337.
Paredes, R.G., Agmo, A., 2004. Has dopamine a physiological role in the control of sexual behavior? A critical review of the evidence. Prog. Neurobiol. 73, 179–226. https://doi.org/10.1016/j.pneurobio.2004.05.001.
Paredes, R.G., Vazquez, B., 1999. What do female rats like about sex? Paced mating. Behav. Brain Res. 105, 117–127. https://doi.org/10.1016/S0166-4328(99)00087-X.
Perlman, W.R., Webster, M.J., Kleinman, J.E., Weickert, C.S., 2004. Reduced glucocorticoid and estrogen receptor alpha messenger ribonucleic acid levels in the amygdala of patients with major mental illness. Biol. Psychiatry 56, 844–852. https://doi.org/10.1016/j.biopsych.2004.09.006.
Perlman, W.R., Tomaskovic-Crook, E., Montague, D.M., Webster, M.J., Rubinow, D.R., Kleinman, J.E., Weickert, C.S., 2005. Alteration in estrogen receptor alpha mRNA levels in frontal cortex and hippocampus of patients with major mental illness. Biol. Psychiatry 58, 812–824. https://doi.org/10.1016/j.biopsych.2005.04.047.
Pfaus, J.G., 2009. Pathways of sexual desire. J. Sex. Med. 6, 1506–1533. https://doi.org/10.1111/j.1743-6109.2009.01309.x.
Pfaus, J.G., Phillips, A.G., 1991. Role of dopamine in anticipatory and consummatory aspects of sexual behavior in the male rat. Behav. Neurosci. 105, 727–743. https://doi.org/10.1037/0735-7044.105.5.727.
Pleim, E.T., Matochik, J.A., Barfield, R.J., Auerbach, S.B., 1990. Correlation of dopamine release in the nucleus accumbens with masculine sexual behavior in rats. Brain Res. 524, 160–163. https://doi.org/10.1016/0006-8993(90)90507-8.
Rodríguez-Manzo, G., 1999. Yohimbine interacts with the dopaminergic system to reverse sexual satiation: further evidence for a role of sexual motivation in sexual exhaustion. Eur. J. Pharmacol. 372, 1–8. https://doi.org/10.1016/S0014-2999(99)00140-5.
Salgado, S., Kaplitt, M.G., 2015. The nucleus accumbens: a comprehensive review. Stereotact. Funct. Neurosurg. 93, 75–93. https://doi.org/10.1159/000368279.
Satou, M., Yamanouchi, K., 1999. Effect of direct application of estrogen aimed at lateral septum or dorsal raphe nucleus on lordosis behavior: regional and sexual differences in rats. Neuroendocrinology 69, 446–452. https://doi.org/10.1159/000054448.
Seifert, U., Härtig, W., Grosche, J., Brückner, G., Riedel, A., Brauer, K., 1998. Axonal expression sites of tyrosine hydroxylase, calretinin- and calbindin-immunoreactivity in striato-pallidal and septal nuclei of the rat brain: a double-immunolabelling study. Brain Res. 795, 227–246. https://doi.org/10.1016/S0006-8993(98)00298-4.
Sharma, H.R., Thakur, M.K., 2015. Correlation of ERα/ERβ expression with dendritic and behavioural changes in CUMS mice. Physiol. Behav. 145, 71–83. https://doi.org/10.

1016/j.physbeh.2015.03.041.
Sheehan, T.P., Chambers, R.A., Russell, D.S., 2004. Regulation of affect by the lateral septum: implications for neuropsychiatry. Brain Res. Brain Res. Rev. 46, 71–117. https://doi.org/10.1016/j.brainresrev.2004.04.009.
Shughrue, P.J., Merchenthaler, I., 2001. Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system. J. Comp. Neurol. 436, 64–81. https://doi.org/10.1002/cne.1054.
Shughrue, P.J., Scrimo, P.J., Merchenthaler, I., 1998. Evidence for the colocalization of estrogen receptor-beta mRNA and estrogen receptor-alpha immunoreactivity in neurons of the rat forebrain. Endocrinology 139, 5267–5270. https://doi.org/10.1210/endo.139.12.6525.
Simerly, R.B., Chang, C., Muramatsu, M., Swanson, L.W., 1990. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J. Comp. Neurol. 294, 76–95. https://doi.org/10.1002/cne.902940107.
Spiteri, T., Ågmo, A., 2006. Modèles précliniques du désir sexuel. Sexologies 15, 241–249. https://doi.org/10.1016/j.sexol.2006.05.001.
Thompson, T.L., Certain, M.E., 2005. Estrogen mediated inhibition of dopamine transport in the striatum: regulation by Gαi/o. Eur. J. Pharmacol. 511, 121–126. https://doi.org/10.1016/j.ejphar.2005.02.005.
Vázquez-Palacios, G., Bonilla-Jaime, H., Velázquez-Moctezuma, J., 2005. Antidepressant effects of nicotine and fluoxetine in an animal model of depression induced by neonatal treatment with clomipramine. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 29, 39–46. https://doi.org/10.1016/j.pnpbp.2004.08.008.
Vega Matuszcyk, J., Larsson, K., Eriksson, E., 1998. The selective serotonin reuptake inhibitor fluoxetine reduces sexual motivation in male rats. Pharmacol. Biochem. Behav. 60, 527–532. https://doi.org/10.1016/S0091-3057(98)00010-0.
Vijayakumar, M., Meti, B.L., 1999. Alterations in the levels of monoamines in discrete brain regions of clomipramine-induced animal model of endogenous depression. Neurochem. Res. 24, 345–349. https://doi.org/10.1023/A:1020992314534.
Vogel, G., Hartley, P., Neill, D., Hagler, M., Kors, D., 1988. Animal depression model by neonatal clomipramine: reduction of shock induced aggression. Pharmacol. Biochem. Behav. 31, 103–106. https://doi.org/10.1016/0091-3057(88)90319-X.
Vogel, G., Neill, D., Hagler, M., Kors, D., 1990. A new animal model of endogenous depression: a summary of present findings. Neurosci. Biobehav. Rev. 14, 85–91. https://doi.org/10.1016/S0149-7634(05)80164-2.
Vogel, G., Hagler, M., Hennessey, A., Richard, C., 1996. Dose-dependent decrements in adult male rat sexual behavior after neonatal clorimipramine treatment. Pharmacol. Biochem. Behav. 54, 605–609. https://doi.org/10.1016/0091-3057(95)02276-7.
Wersinger, S.R., Rissman, E.F., 2000. Dopamine activates masculine sexual behavior independent of the estrogen receptor alpha. J. Neurosci. 20, 4248–4254. https://doi.org/10.1523/JNEUROSCI.20-11-04248.2000.
Willner, P., Mitchell, P.J., 2002. The validity of animal models of predisposition to depression. Behav. Pharmacol. 13, 169–188. https://doi.org/10.1097/00008877-200205000-00001.
Wood, R.I., Newman, S.W., 1995. Androgen and estrogen receptors coexist within individual neurons in the brain of the Syrian hamster. Neuroendocrinology 62, 487–497. https://doi.org/10.1159/000127039.
Yamanouchi, K., 1997. In: Maeda, K.I., Tsukamura, H., Yokoyama, A. (Eds.), Brain mechanisms inhibiting the expression of heterotypical sexual behavior in rats. Japan Scientific Societies Press, Tokyo, pp. 219–235.
Yoest, K.E., Cummings, J.A., Becker, J.B., 2014. Estradiol, dopamine and motivation. Cent. Nerv. Syst. Agents Med. Chem. 14, 83–89. https://doi.org/10.2174/1871524914666141226103135.