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Maternal exposure to the antidepressant fluoxetine impairs sexual motivation in adult male mice

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ABSTRACT

Depressive disorders have a worldwide high prevalence. Fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI) antidepressant, has been widely prescribed for depression during pregnancy and/or lactation. Since serotonin is a neurotrophic factor, the use of FLX by mothers could disrupt brain development resulting in behavioral alterations in their progeny. The aim of the present study was to evaluate the effects of developmental FLX exposure on sexual behavior, as well as on endocrine parameters, of male mice. Swiss dams were treated daily, by gavage, with 7.5 mg/kg of FLX during pregnancy and lactation. Male pups were tested for copulatory behavior and sexual incentive motivation. Male pups also had their anogenital distance, plasmatic testosterone concentration and testis, epididymis, seminal vesicle and pituitary wet weights assessed. Copulatory behavior, anogenital distance, plasmatic testosterone concentration and organs wet weights were not affected by FLX exposure. However, this exposure eliminated preference for a sexual incentive on the sexual incentive motivation test, which indicates reduced sexual motivation, a classic side effect of SSRIs in humans who take these antidepressants.

1. Introduction

Depression is common, affecting about 121 million people worldwide (World Health Organization, 2007). Epidemiological studies indicate that lifetime risk for depression ranges between 10 and 25% for women, with a peak prevalence occurring between the childbearing ages of 25–44 years (Burt and Stein, 2002; Sloan and Kornstein, 2003). Approximately 10% of women will suffer from psychological distress during pregnancy (McElhatton, 2003), and evidence suggests that maternal depression during pregnancy is at least as common as postpartum depression (Evans et al., 2001). In some women, treatment with antidepressants cannot be avoided. The decision of prescribing and taking psychotropic drugs during gestation and lactation is a difficult task due to the paucity of studies investigating the safety of these drugs for the babies, especially for their long-term neurobehavioral development (McElhatton, 2003; Zeskind and Stephens, 2004). Among antidepressants, fluoxetine (FLX), a selective serotonin (5-HT) reuptake inhibitor (SSRI) drug, has been widely prescribed for depression during pregnancy and lactation due to its high degree of selectivity and its minimal side effects compared with tricyclic and monoamine oxidase inhibitor antidepressants (American Academy of Pediatrics, 2000; Einarson and Koren, 2004). However, since FLX readily crosses the placental barrier (Pohland et al., 1989) and is excreted in milk (Hendrick et al., 2001), fetuses and newborns of mothers who take this antidepressant are exposed to increased 5-HT levels during early brain development.

During embryogenesis, 5-HT regulates the development of γ-aminobutyric acid and monoamine systems and is involved in cell migration, axon growth and synaptogenesis (Lauder, 1993; Whitaker-Azmitia et al., 1996). In rats, increased prenatal levels of 5-HT produced adverse effects, including reduction of phosphoinositide hydrolysis induced by 5-HT receptor stimulation (Romero et al., 1994), reduced numbers of 5-HT transporters (Montero et al., 1990) as well as 5-HT and β-adrenergic receptors and abnormalities in the brain 5-HT receptor binding (De Ceballos et al., 1985; Jason et al., 1981).

The brain sexual phenotype of a developing fetus is essentially undifferentiated and bipotential, being determined by exposure to sexual hormones during an early critical period. As revised by Wilson and Davies (2007), the hormone-induced brain sexual differentiation is in part mediated by neurotransmitters, including 5-HT. González and Leret (1992) observed a long-term striatal and limbic increase in 5-HT

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metabolism induced by early androgen-derived estradiol action. Neonatal 5-HT antagonizes testosterone masculinizing effects on size of sexually dimorphic hypothalamic nuclei and gonadotropin release (Murray et al., 2004), and 5-HT hypothalamic concentration is reduced in critical periods of male rat brain masculinization (Ladosky and Gaziri, 1970; Giulian et al., 1973), probably to allow adequate testosterone action.

This study was carried out in mice in order to evaluate if maternal exposure to FLX during pregnancy and lactation could disrupt sexual behavior and induce endocrine alterations in the progeny.

2. Materials and methods

2.1. Animals and experimental protocol

Male and female Swiss mice (70–90 days) from the colony of the State University of Londrina (UEL) were used as parent generation. They were kept in a controlled environment with temperature at 25±1 °C; humidity of 55±5%; 12 h light/dark cycle (lights on at 6:00 a.m.) and had free access to regular lab chow and tap water.

Mice were mated (2 females and 1 male per cage) and gestational day (GD) 0 was determined by the presence of a vaginal plug. Dams were divided into two groups:

- Control group (CON): 19 dams received daily 0.25 ml of tap water (by oral gavage) from GD 0 to post-natal day (PND) 21.
- Fluoxetine group (FLX): 20 dams received daily 7.5 mg/kg of FLX (Daforin® liquid, Novaquimica, Brazil) by oral gavage from GD 0 to PND21 (Lisboa et al., 2007). The dose of FLX was based on the weight of dams on GD 0, and was not corrected according to weight fluctuations during gestational and lactational periods, since this correction is not carried out in humans.

At birth (PND1), all litters were weighed and on PND 4 they were culled to 8 pups. Whenever possible, an equal number of male and female pups was kept within the litter. Pups were weaned on PND 21.

All the experimental protocol was approved by the State University of Londrina Ethics Committee for Animal Research (protocol number: CEEA 24/05).

2.2. Behavioral evaluation

Sexual behavior was observed in adult mice (PND 90±10), during the dark phase of a reverse light/dark cycle, under dim red light. The animals were allowed a 15-day period of adaptation to reverse light/dark cycle before the beginning of the experiments. The observations always started 4 h after the onset of darkness and were recorded by a video camera, linked to a monitor in an adjacent room. For behavioral evaluation, only one pup from each litter was used, i.e., the litter was the experimental unity (Organization for Economics Cooperation and Development, 2006).

2.2.1. Copulatory behavior

Sexually naive male pups (7 males from control group and 9 males from fluoxetine group) were placed into a Plexiglas cage and, after 10 min, a naturally receptive female was introduced. During 30 min, the latencies and numbers of mounts, intromissions and ejaculations were observed as described previously (Gerardin et al., 2006).

2.2.2. Sexual incentive motivation

The same animals (6 males from control group and 9 males from fluoxetine group) evaluated for copulatory behavior were used for the sexual incentive motivation test. The cage consisted of a plastic rectangular arena (40×32 cm). On each long wall, an opening (10×10 cm) was located and faced an incentive animal cage (10×10×10 cm) in which one incentive animal was placed. The two openings were diagonally opposed and covered with wire mesh. The incentives used were an estrous female in one cage (sexual incentive) and a sexually active male in the other cage (social incentive). A zone (9×14 cm) outside each incentive animal cage was designated the incentive zone. The experimental male was placed in the center of the arena and observed for 20 min. The number of visits and the total time spent visiting each incentive zone were quantified, and a preference score (time spent in female zone/total time spent in both incentive zones) was calculated (Agmo, 2003).

2.3. Endrocrine parameters

2.3.1. Anogenital distance

At birth (PND 1) and on PND 120 the anogenital distances were obtained through a vernier caliper. On PND 1 data are expressed as litter mean and on PND 120, they are individual data.

2.3.2. Organs wet weights

Two weeks after behavioral evaluation, male pups (7 males from control group and 9 males from fluoxetine group) were weighed and anaesthetized with sodium pentobarbital (40 mg/kg, ip). Blood samples were collected and one testis, one epididymis, one seminal vesicle and pituitary were removed for wet weight determination.

2.3.3. Plasmatic testosterone

Blood samples (7 males from control group and 9 males from fluoxetine group) were collected by intracardiac puncture and blood samples were immediately placed in heparinized vials which were kept on ice until centrifuged (2500 rpm for 20 min at 2 °C). The plasmatic testosterone concentration was assayed by chemoluminescence technique.

2.4. Statistical analysis

Preference scores from sexual incentive motivation test were compared with chance preference (i.e., a score of 0.5) by One sample Student's t test (H_0: $m=0.5$). The remaining data were evaluated by Student's t test with Welch's correction. Differences were considered significant if $p<0.05$.

3. Results

3.1. Body weight and anogenital distance

Student's t test with Welch's correction showed that FLX exposure did not alter anogenital distance or body weight at birth as well as on adulthood (Table 1).

3.2. Behavioral evaluation

In the copulatory behavior test, none of the animals ejaculated within the 30 min of test. Student's t test showed that FLX exposure did not alter any of the observed behavioral parameters (Table 2).

Table 1
Anogenital distance and body weight of male mice at birth and on PND 120

	Anogenital distance (mm)		Body weight (g)	
	CON	FLX	CON	FLX
PND 1	2.48±0.04 (19)	2.46±0.03 (20)	2.9±0.2 (19)	2.9±0.1 (20)
PND 120	18.90±0.62 (7)	18.36±0.48 (9)	44.9±1.1 (7)	45.2±1.7 (9)

Number in parentheses represent the number of litters (PND1) or animals (PND120) per group. Data are means±SEM. Student's t test with Welch's correction ($p>0.05$).

Table 2
Copulatory behavior of adult male mice

	CON (n=7)	FLX (n=9)
Mount latency (s)	281.6±39.4	379.8±85.3
Intromission latency (s)	420.6±75.5	453.7±57.1
Number of mounts	5.6±1.2	4.9±0.9
Number of intromissions	32.8±4.8	27.4±3.7

Data are means±SEM. Student's t test with Welch's correction (p>0.05).

In the sexual incentive motivation test, Student's t test showed that, compared to CON, FLX-exposed males visited more ($t(10)=2.3$, $p=0.04$) and spent more time ($t(11)=3.1$, $p=0.01$) in the social zone (that contained an adult male) (Table 3). As expected one sample Student's t test showed that the preference score of the CON group was significantly different of 0.5 ($t(5)=4.6$, $p=0.006$), i.e., the time spent within the sexual zone (containing a female) was significantly higher than the time spent within the social zone (containing a male). On the other hand, in the FLX-exposed group, the preference score was statistically undistinguishable of 0.5, i.e., the animals spent equivalent times within both zones, having no preference by the sexual zone (Table 3).

3.3. Organs wet weights and plasmatic testosterone

Student's t test showed that FLX exposure did not influence testis, epididymis, seminal vesicle and pituitary wet weight (Fig. 1), as well as mean plasmatic testosterone concentration (CON: 816.29±187.75; FLX:1064.11±256.65 ng/dL).

4. Discussion

The present study evaluated the effects of FLX exposure during pregnancy and lactation on sexual behavior and endocrine aspects of male pups. The regimen of exposure adopted in the present study has been used in our laboratory and it does not induce maternal toxicity or litter size and weight alterations (Lisboa et al., 2007), which could be responsible for occasional behavioral alterations observed in pups.

Anogenital distance is a sexually dimorphic parameter. Alterations are believed to be due to an inadequate action of testosterone and could be an early indicator of impaired sexual activity at adulthood (Keshet and Weinstock, 1995). FLX exposure did not alter anogenital distance at birth or adulthood as well as other endocrine aspects, such as plasmatic testosterone concentration and wet weights of testis, epididymis, seminal vesicle and pituitary. These observations suggest that, in male mice, developmental FLX exposure does not disrupt gonadal hormones function.

Regarding behavioral evaluation, FLX exposure did not alter any of the parameters assessed on the copulatory behavior test. On the other hand, alterations in sexual incentive motivation test were observed. In this test, the percentage of time spent in the vicinity of an estrous female has been regarded as a measure of sexual motivation, a putative correlate to human libido (Matuszcyk et al., 1998; Agmo 1999, 2003; Portillo and Paredes, 2004). As expected, CON animals spent significant more time in the sexual zone. However, FLX-exposed males explored both social and sexual zones indiscriminately, resulting in no sexual preference and suggesting that developmental FLX exposure reduced sexual motivation. Reduced libido is a classic adverse effect of chronic FLX treatment in humans (Gregorian et al., 2002; Clayton et al., 2006), and was already observed in adult rats chronically exposed to FLX (Matuszcyk et al., 1998). The novelty of our data is to suggest long-lasting reduced sexual motivation in male pups exposed to FLX during neurodevelopment.

Table 3
Sexual incentive motivation test of adult male mice

	CON (n=6)	FLX (n=9)
Time spent in male zone	236.8±17.8	353.1±33.4*
Time spent in female zone	439.8±32.2	436.2±30.6
Number of visits in male zone	29.0±2.9	37.4±2.2*
Number of visits in female zone	36.2±3.0	42.1±3.0
Preference score	0.65±0.03†	0.55±0.03

Data are means±SEM.
*p<0.05 compared to CON group (Student's t test with Welch's correction).
†p<0.01 (One sample Student's t test, H_0: m=0.5).

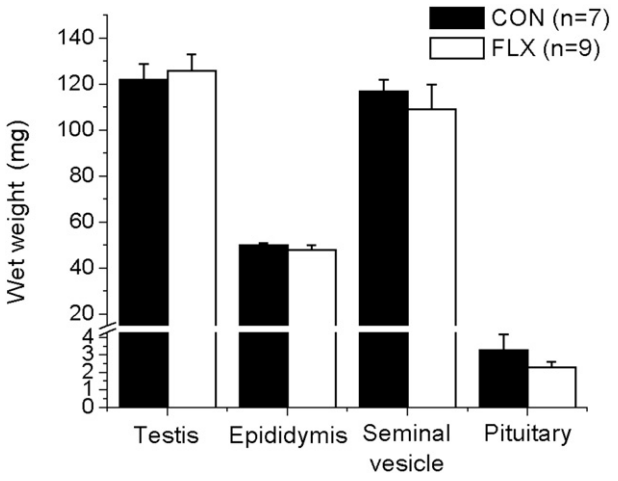

Fig. 1. Wet weight of organs of male mice at PND 120. Data are means±SEM. Student's t test with Welch's correction (p>0.05).

Traditionally, dopamine (DA) has been linked to sexual motivation (Everitt, 1990; Hull et al., 2004; Dominguez and Hull, 2005) and unpublished data from our laboratory suggests that the same regimen of exposure to FLX adopted in the present study seems to disrupt dopaminergic neurotransmission, which could play a role on the decreased sexual motivation observed. However, it has been suggested that DA is not the neurotransmitter involved in the sexual motivation evaluated in the test employed in the present study (Agmo, 2003; Paredes and Agmo, 2004) and that serotonergic neurotransmission could be involved. In fact, administration of 5-HT_{2A} receptor antagonists (ketanserin or cyproheptadine) in adult mice diminished time spent in the sexual zone (Popova and Amstislavskaya, 2002). This same effect was also observed after neonatal treatment with the 5-HT_2 agonist DOI in rats (González et al., 1996), whereas an opposite effect (i.e. increased sexual motivation) was observed after neonatal inhibition of 5-HT synthesis (Farabollini et al., 1988). Taken together, these data indicate the importance of adequate serotonergic neurotransmission mediated by 5-HT_2 receptor on the sexual motivation. Interestingly, the reduced number of 5-HT_2 receptors has been described in the hypothalamus of rats after prenatal FLX exposure (Cabrera and Battaglia, 1994) and in the frontal cortex of prepubertal rat after prenatal tryciclic antidepressant exposure (De Ceballos et al., 1985). If long-term 5-HT_2 receptor downregulation after maternal FLX exposure is involved in the reduced sexual motivation observed in the present study remains to be investigated in future researches.

In conclusion, our data suggest that maternal FLX exposure has an enduring negative influence on the sexual motivation of male mice descendants, bringing about the necessity of more researches about the effects of maternal exposure to selective serotonin reuptake inhibitor antidepressants during critical periods of brain development.

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