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Neurofunctional effects in rats prenatally exposed to fluoxetine

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Abstract. – In the treatment of depression fluoxetine [a selective serotonine reuptake inhibitor (SSRIs)] is a widely used drug in humans. The selectivity, efficacy, side effects and simplicity of dosage contributed to fluoxetine's clinical acceptance. Several psychiatric disorders (many of them responsive to SSRIs) are present during pregnancy; up to 10% of pregnant women fulfill diagnostic criteria for major or minor depression with an even higher percentage developing postpartum depression. Therefore, significant numbers of women may be taking SSRIs while pregnant. Since fluoxetine's safe use during pregnancy is not yet established and experimental studies inconclusive, we performed the present research in order to investigate the neurobehavioral effects produced in rats by prenatal exposure to fluoxetine (5 and 10 mg/kg/sc from day 13 to 20 of gestation) on cognitive functions, emotional reactivity and sexual performance.

Key Words:

Fluoxetine, SSRIs, Rat, Pregnancy, Sexual behaviour, Emotionality, Learning, Memory.

Abbreviatons

GD = Gestational day
PND = Postnatal day
CS = Conditioned stimulus
US = Unconditioned stimulus
F5 = Fluoxetine 5 mg/Kg
F10 = Fluoxetine 10 mg/kg
M/IL = Mount/intromission latency
M/IF = Mount/intromission frequency
EjL = Ejaculation latency
EjF = Ejaculation frequency
PEjI = Post-ejaculatory interval
L50 = Latency to the first 50 kHz call
L22 = Latency to the first 22 kHz call
D22 = Duration of the post-ejaculatory 22 kHz vocalization

Introduction

Starting from 1988, fluoxetine has been the first selective serotonine reuptake inhibitor (SSRIs) effective in the treatment of depression. Among the serotonin (5-HT) uptake inhibitors, fluoxetine seems to possess the lowest rate of side effects in humans. These characteristics (selectivity, efficacy, lower side effects and simplicity of dosage) have contributed to fluoxetine's clinical acceptance[1]. Fluoxetine in UK has been the principal SSRIs prescribed for depression[2]. The clinical efficacy of several SSRIs like sertraline, zimelidine, fluoxetine, fluvoxamine and paroxetine were compared with some classic tricyclic antidepressants whose efficacy or onset of action have a different side effects potential. SSRIs resulted unable to replace tricyclics antidepressants but had a useful addition to various drugs currently used for the treatment of depres-

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sion[3]. SSRIs and tricyclics are frequently used by women of reproductive age, since several major psychiatric disorders could be present during this time period. While pregnant, a significant number of women may be taking SSRIs for major or minor depression and up to 10% of pregnant women fulfill diagnostic criteria with an even high percentage in developing postpartum depressions[4-6]. In addition, mood disorders and anxiety disorders, usually present in women during their childbearing years, increase the potential need of an antidepressant therapy during pregnancy[7].

Several studies, performed in socially disadvantaged populations, reported a relationship between an increased risk for obstetric complications and maternal depression together with preterm birth and/or small offsprings for their related gestational age[8-18]. Some recent reviews suggest that fluoxetine, or SSRIs group, are actually considered the first line of treatment of depression during pregnancy[19-21].

Due to a lower ability to be metabolized and to a lower capacity of glomerular filtration rate, psychotropic drugs quickly trespass the placenta and their fetal metabolites could result higher than the level found into maternal circulation[22]. Caused by the immaturity of the fetal blood-brain-barrier, an high level of psychotropic drugs could be also found in the fetal brain[23]. Blood samples in umbilical cord at delivery also resulted to contain antidepressants and metabolites[24].

Moreover, fluoxetine resulted to easy cross the placenta with subsequent fetal distribution during and after organogenesis with the highest concentration of the radiolabeled fluoxetine localized in the fetal brain and thymus[25].

In addition, clinical evaluation of exposed fetuses to fluoxetine during the third trimester of pregnancy (but not limited to), showed postnatal complications including irritability, jitteriness, sleep disturbance, palpitations and premature delivery[26]. A clinical study regarding the exposure during pregnancy demonstrated, in the newborn exposed to fluoxetine during the last trimester, a slight delays in psychomotor development and in motor movement control[27] with an associated higher rate of prematurity, a lower APGAR score, a low-birth weight, an admission to special care nurseries (SCNs) and a "poor neonatal adaptation"[28-30]. Another clinical study, focused on the safe use of SSRIs during the last trimester of pregnancy, found a neonatal withdrawal syndrome occurring after in utero exposure to SSRIs.

It was concluded that neonates, exposed to SSRIs during the last trimester of pregnancy, should be closely followed-up for after birth withdrawal symptoms[31]; on the contrary, other clinical studies focused on the use of SSRIs during the first trimester of pregnancy seemed not to be associated with measurable teratogenic effects in humans[32] and that, in utero exposure to antidepressant drugs, does not affect global IQ, language development or behavioral development in preschool children[33].

Due to a poor number of informations available from prospective studies providing changes from decision based on non-prospective data, it was strictly recommended monitoring and interventions for patients with identified risks (e.g., poor weight gain)[34]. Therefore, a recent clinical study evidenced an increased risk of low birth weight, jaundice and respiratory distress in infants born from mothers perinatally exposed to SSRIs[35].

A large number of experimental studies have been reported on the animal exposure to fluoxetine during pregnancy. One research demonstrated a prolonged 30% down-regulation of [^{3}H]imipramine binding sites in the cerebral cortex of rats exposed to fluoxetine during pregnancy. Conversely, the 5-HT disruptions were not seen in adult rats treated with fluoxetine suggesting that the rat developing brain is particularly sensitive to the antidepressants[36]. Fluoxetine, administered in rats during pregnancy, induced a decrease of the body weight in both sexes at birth and in 70 days old males. Moreover, it was also found a 35% reduction in hypothalamic 5-$HT_{2A/2C}$ receptor density not fully evident until puberty. It was hypothesized that an hormonal change, related with the animal age, could be involved in the expression of such effects; functional changes of the serotonergic system were also seen: a significant (58%) reduction in the elevation of plasma adrenocorticotropic hormone was also found in response to a 5-$HT_{2A/2C}$ agonist[37]. In addition, it was demonstrated that prenatal exposure to fluoxetine produced in brain 5-HT neurons and 5-HT transporters of male rat offspring, region-specific and age-dependent changes[38,39]. *In vitro* exposure studies to sertraline, fluoxetine and amitriptyline on mouse embryos, produced craniofacial malformations[40]. These data were also supported by a study on the role of serotonin during the mammalian craniofacial development[41]. In another study, it was also found that the prenatal exposure to fluoxetine af-

fected birth weight and sex of rats also reducing the duration of pregnancy[42]. Furthermore, an increased incidence of skin haematomas was found in pregnant mice exposed to fluoxetine from day 7 of gestation up to delivery[43].

On the basis of the above exposed clinical and experimental data, the aim of the present study is to investigate the cognitive functions, the emotional reactivity and the sexual performance of adult rats prenatally exposed to fluoxetine (5, 10 mg/kg) during the last third of pregnancy.

Materials and Methods

Animals and Exposure Conditions

The experiments have been conducted in accordance with the guidelines released by *Italian Ministry of Health* (D.L. 116/92), the *Declaration of Helsinki* and the "*Guide for the Care and Use of Laboratory Animals*", as adopted and promulgated by the *National Institute of Health* (Bethesda, Maryland, USA).

Primiparous Wistar female rats (Harlan, Italy) weighing 250-280 g were used. Animals were allowed free access to food and water, were housed at constant room temperature (20-22°C) and exposed to a light cycle of 12 h/day (08:00 h-20:00 h) for 2 weeks before the experiment. Pairs of females were placed with single male rat in the late afternoon. Vaginal smears were taken the following morning at 09:00 h. The day on which sperm were present was designated as day 0 of gestation (GD 0). Pregnant rats were then randomly assigned to three experimental groups (vehicle, fluoxetine hydrochloride 5 mg/kg/2 ml (F5) and 10 mg/kg/2 ml (F10), s.c.). Once daily (at 9.00 a.m.), beginning on GD 13 and ending on GD 20, experimental dams received subcutaneous injections of either saline (2 ml/kg) or fluoxetine-HCl dissolved in 2 ml/kg of saline. Fluoxetine was obtained by courtesy of Eli Lilly Italia S.p.A., (Firenze, Italy). All litters were reduced to a standard size of four male and four female pups per litter (when possible) within 24 h after birth. Litters from the control group (vehicle) or fluoxetine-treated groups were then assigned (eight pups per litter) to non-treated mothers, whose pups were born on the same day. Experimental pups were then weaned at 21 days of age. One male pup per litter from different litters per treatment group was used in behavioural experiments.

Reproduction Data

The body weights of dams (Vehicle group: 10 dams; F5 group: 11 dams; F10 group 11 dams) were taken on day 0 and from 13 to 20 days of gestation. For each group were detected and analized the number of male and females/litter, gestation length (days), dams giving birth (%), pregnancy length (days), dams weight gain (%), litter size at birth (n), postnatal mortality (%), male and female birth-weight/litter (g), pups dead/born at birth (n[%]) and pups died before weaning (postnatal mortality) (%). All pups were sexed and weighted on postnatal day one (PND 1) and examined for the presence of external anatomical malformations. Afterwards, animals were weighted on PNDs 7, 13, 21, 30, 40, 60, 80 and 120.

Homing Behavior and Related Locomotor Activity

The procedure and the apparatus of this test have been previously described by Altman and Sudarshan[44]. Briefly, the apparatus consisted of a central circular area, 11 cm in diameter, surrounded by a wire fence. The orientating platform was positioned between two enclosed alleys, 7.5 cm long, which opened into the home cage (containing mother and siblings) on one side and into an empty cage on the opposite side. The size of the fenced-in area limited pup locomotion and, to facilitate scoring, the circular area was divided by octagonally oriented lines.

The animal was randomly placed facing right or left at a right angle to the entrances; these were the null points. Turning toward the home cage or empty cage was scored as +1 or –1, and turning half-way to the two entrances was scored as +1/2 or –1/2.

Each male pup was individually removed from the home cage and placed into the fenced-in circle for 180 sec. and its orientation was recorded every 10 sec. For each animal, scores were summed and the mean score of all animals was calculated (maximum individual score was +18 if the animal was oriented at all observation periods fully towards the opening of the home cage). Because homing performance could be influenced by the level of locomotor activity, this parameter was also evaluated. To score their locomotor activity, the fenced-in area was divided in nine identical surfaces (eight circular sectors surrounding a central circular area) and the number of lines crossed by each rat was measured during the test session (crossings). This test was carried

out on PNDs 6-10 and one male pup per litter from different litters in each experimental group was used (vehicle: 10 pups; F5 11 pups; F10 pups).

Ultrasonic Vocalization in Rat Pups

Recording sessions were conducted in a sound-attenuating chamber (3.00 × 2.00 × 2.25 m). Vocalizations of rat pups were detected by an ultrasonic microphone connected to a receiver (Bat Detector S25 (Ultra Sound Advice, UK) which transformed, in real time, the ultrasonic calls into audible sounds. Microphone signals were also relayed, through the high frequency output socket of the Bat Detector, to a Racal Store 4DS high-speed tape-recorder (HSTR) using a direct mode recording procedure running at a tape speed of 30 i.p.s. (76.8 cm/s). The rate of the ultrasonic vocalizations (no. of calls/15 s), emitted by male rat pups at 10 days of age, was later analyzed reducing the tape speed of the HSTR from the original 30 i.p.s. (76.2 cm/s) to 7.5 i.p.s. (19.05 cm/s) and simply counting the ultrasonic emissions listening to a loudspeaker connected to the output of the HSTR and visualizing them, in intensity-time mode, on the screen of a Bruel & Kjaer High Resolution Signal Analyzer.

From each litter of the experimental groups one male pup was removed from the nest and placed in a shallow plastic dish (15 cm in diameter and 6 cm deep) 15 s before the test. This confined the pup movement relative to the microphone which was supported vertically 15 cm above the dish and thus avoiding to handle them during the recording session which lasted 15 s.

Motor Activity

Motor activity was recorded in Macrolon cages by infrared monitoring beams, according to the technique previously described by Tamborini et al.[45] and modified as follows. Briefly, a passive infrared sensor (RK 2000 QPC) was placed 23 cm above the center of each cage (59 × 38.5 × 20 cm) which was covered with stainless steel wire lids. The infrared sensors were connected, via an interface, to a Personal Computer whose software was scheduled to check all sensors at 1-s intervals. A sensor which was registering no movement gave a 0 score for that interval and a sensor which was registering a movement, gave a 1 score for that interval. These scores were then summed and the calculation performed over 15-min blocks. Therefore, the theoretical range of activity for 5 min was 0-300. All tests were carried out in a 3 × 2 × 2 m sound attenuating cabin illuminated by a 20-W white light. Background noise of 46 dB was produced by a fan. Animals were subjected to 1 hour session at 21 and 60 days of age. Each test started when an animal was placed in the center of the arena. Immediately after each test, the apparatus was thoroughly cleaned by cotton pads wetted with 96% ethanol solution. At 21 days of age the following male pups were tested: vehicle = 8 pups; F5 = 8 pups; F10 = 8 pups; at 60 days of age were tested: vehicle = 8 pups; F5 = 8 pups; F10 = 7 pups.

Active Avoidance Conditioning and Related Ultrasonic Vocalization

The apparatus and the procedure have been previously described by Cuomo et al.[46] and modified by De Salvia et al.[47]. Briefly, the apparatus consisted of a two-way avoidance box housed inside a 3 × 2 × 2 m sound attenuating cabin. Each avoidance box was divided into two compartments connected by an opening of 9 × 12 cm and operated by an electromechanical programming equipment. The conditioned stimulus (CS) consisted of a light (3-W lamp) which was alternately switched-on in each compartment. The onset of the CS was followed 12 s later by the onset of an unconditioned stimulus (US), which was a 0.8 mA- scrambled foot shock. The CS remained on during the presentation of the US, which lasted a maximum of 18 s, afterwards both CS and US were turned off. A conditioned avoidance response (CAR) was recorded when an animal avoided the US by crossing over to the opposite compartment during the first 12 s when only the CS was on. An escape response consisted of the animal moving into the opposite compartment following the onset of the US. This response terminated both the CS and the US. Sixty-day-old male rats were subjected to 75 trials session (three 25-trial blocks) with a 60 s intertrial interval (ITI). Ultrasonic calls (UC) emitted during the presentation of the CS or during the ITI were recorded by a QMC ultrasonic microphone (placed at the center of the shuttle box cover) connected to a receiver (QMC Bat Detector S200) which transformed, in real time, the UC into audible sounds. Microphone signals were relayed, via the high frequency output socket, to a Bruel & Kjaer (2033) High Resolution Signal Analyzer (HRSA) set on time-intensity mode in order to visualise the ultrasonic calls. The num-

ber of UC emitted by rats (22 KHz calls) was counted manually by listening to the audible output of a headphone. The shuttle-box recording unit signalled the period (CS or ITI) in which ultrasounds occurred. The following 60 day old male rats were used in this experimental procedure: vehicle = 10 rats; F5 = 10 rats; F10 = 7 rats.

Passive Avoidance Test

A "step-down" apparatus was used. It consisted of a compartment (25 × 24 × 24 cm) constructed of black Plexiglas and equipped with a grid floor to which an elevated compartment (13 × 24 × 16 cm) with a solid Plexiglas floor was attached. A guillotine door (9 × 10 cm) separated the opening between the elevated compartment and the large compartment. A 25-W lamp illuminated the elevated compartment while the large compartment remained dark. Scrambled foot shocks were delivered from a Letica shock generator (LI 2750 Unit, Barcelona, Spain). The experiments were performed in a sound-attenuating chamber (Amplifon G-type cabin) that was dark except for illumination of the elevated compartment of the apparatus.

Procedure. Each animal was removed from the home cage and placed in a holding cage adjacent to the apparatus. Two minutes later, the rat was placed in the illuminated compartment, and, after a 10-s delay, the guillotine door was raised. The time taken by the animal to completely enter into the dark compartment was measured (approach latency) and taken as an index of emotional, nonassociative behavior. A single 2-s inescapable scrambled foot shock (0.8 mA) was delivered immediately after the rat entered the dark compartment. Twenty-four hours after this session (acquisition trial), each animal was tested for memory retention. The animal was placed in the elevated compartment and latency to re-enter (avoidance latency) the dark compartment was recorded and assumed to be a measure of memory retention. Both acquisition and retention trials lasted for a maximum observation time of 180 s. The experiments were conducted in the following 60-day-old male offspring: vehicle = 6 rats; F5 = 6 rats; F10 = 6 rats.

Sexual Behaviour and Related Ultrasonic Vocalization

The technique has been previously described by Cagiano et al.[48]. Heterosexually naive male rats, prenatally exposed to vehicle or fluoxetine, were tested for sexual behaviour at 80 days of age (30 min session). Thereafter, animals were subjected to a further two 30 min sessions (the inter-session interval was 15 days). As stimulus females, we used bilaterally ovariectomized female rats in which oestrous had been induced by subcutaneous injections of estradiol benzoate (8 microg/rat) and progesterone (200 microg/rat) dissolved in 0.2 ml of sesame oil, 52 and 4 h before the test sessions, respectively. Male and female rats were housed under a reversed 12/12 h light-dark cycle (light on: 20.00 h-08.00 h) for 2 weeks before testing. Each male rat was tested for sexual behaviour with a stimulus female under red illumination provided by two 40 W fluorescent lamps. Sexual behaviour was recorded by a JVC video camera connected to a JVC videotape recorder. The experiments, performed in the central part of the dark period (12.00h-16.00h), were carried out in a 3 × 2 × 2 mt sound-attenuating cabin in which a plastic transparent cylinder (diameter = 50 cm; height = 40 cm) was placed on a square transparent glass floor at a height of 130 cm from the floor of the cabin. A 50 cm square mirror was placed at 45° below the floor of the cylinder in order to have the inferior sight of the arena and to record the sexual behaviour with a video-camera placed orizontally outside the cabin, looking the mirror through a sound attenuated glass-camera window. Each male rat was first placed alone into the mating arena and observed along 5 min; an oestrous female was then introduced into the centre of the arena and the behaviour of the male rat was then recorded. Video tape-recordings were later replayed and analysed (in slow motion when necessary) and the following parameters were measured: (M/IL) mount-intromission latency (time between the introduction of the female into the mating cage and the first mount or intromission in each ejaculatory series); (M/IF) mount-intromission frequency (number of mounts or intromissions in each ejaculatory series); (EjL) ejaculation latency (time between the first intromission and ejaculation in each ejaculatory series); (EjF) ejaculation frequency (number of ejaculations in each session); (PEjI) post-ejaculatory interval (interval between each ejaculation and the next intromission in each ejaculatory series). We also evaluated the following ultrasonic parameters:

- (L50) latency from the introduction of the stimulus female into the mating cage and the onset of the first 50 kHz vocalization;

- (L22) time from the ejaculation and the beginning of the 22 kHz vocalization in each ejaculatory series;
- (D22) duration of the 22 kHz post-ejaculatory vocalization in each ejaculatory series.

If no intromission was displayed within the first 15 minutes, the test was over and the male was considered a "non copulator". This test was performed in 80 day-old male offsprings (vehicle = 9 rats; F5 = 9 rats; F10 = 6 rats).

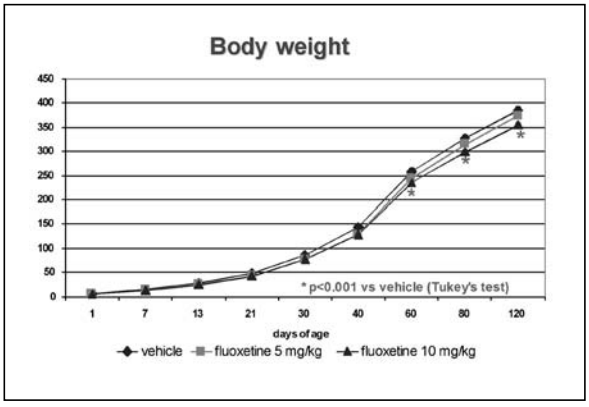

Figure 1. Neonatal weight gain.

Results

Reproduction Data

One-way ANOVA showed that dam weight gain, pregnancy length and litter size at birth were not significantly affected by prenatal treatment with fluoxetine. Moreover, the prenatal treatment with fluoxetine induced a significant decrease of male and female pup weight at birth (male: F = 7.27, df = 2/28, $p < 0.01$; female: F = 6.76, df = 2/28, $p < 0.01$). In particular, a post hoc test (Tukey's test) showed a significant decrease ($p < 0.01$) of this parameter at the highest dose (10 mg/kg) of fluoxetine. Furthermore, Fisher's exact test revealed that fluoxetine exposure induced, at the highest dose, a significant increase of postnatal mortality ($p < 0.001$) together with % of pups dead at birth ($p < 0.001$) (Table I).

Male Pup Weight Gain

Over-all two-way ANOVAs for repeated measures showed that male pup weight gain from PNDs 1 to 120 was significantly affected by prenatal exposure to fluoxetine: (i) between treatments (F = 13.2, df = 2/26, $p < 0.001$.); (ii) between ages (F = 7694, df = 8/208, $p < 0.001$); (iii) between treatments × ages (F = 4.87, df = 16/208, $p < 0.001$). In particular, a post hoc test (Tukey's test) showed a significant decrease ($p < 0.001$) at the highest dose (10 mg/kg) on PNDs 60, 80 and 120 (Figure 1).

Homing Behavior and Related Locomotor Activity

Over-all two-way ANOVAs for repeated measures of orientation scores showed the following results: (i) between treatments ($F_{treatments} = 8.77$ df = 2/49, $p < 0.001$.); (ii) between times ($F_{times} = 88.76$, df = 4/196, $p < 0.001$); (iii) between treatments × times ($F_{treatments \times times} = 2.29$, df = 8/196, $p < 0.05$). However, individual comparisons (Tukey's test) did not show any significant difference.

As far as locomotor activity, one-way ANOVA of the number of crossings showed the following results: $F_{rat\ lines} = 2.80$, df = 1/28, n.s.; $F_{treatments} = 2.60$, df = 1/28, n.s.; $F_{rat\ lines \times treatments} = 2.18$, df = 1/28, n.s.

Ultrasonic Vocalization in Rat Pups

Prenatal exposure to fluoxetine significantly affected the number of ultrasonic calls/15 sec. emitted by 10-day-old male pups removed from their nest. A overall ANOVA showed the following differences between groups: F = 3.93, df = 2/15, $p < 0.05$). Individual comparisons between groups showed that rat pups exposed to fluoxetine (10 mg/kg) during gestation exhibited a significant increase in the rate of ultrasonic calls with respect to controls ($p < 0.05$, Dunnett's test) (Figure 2).

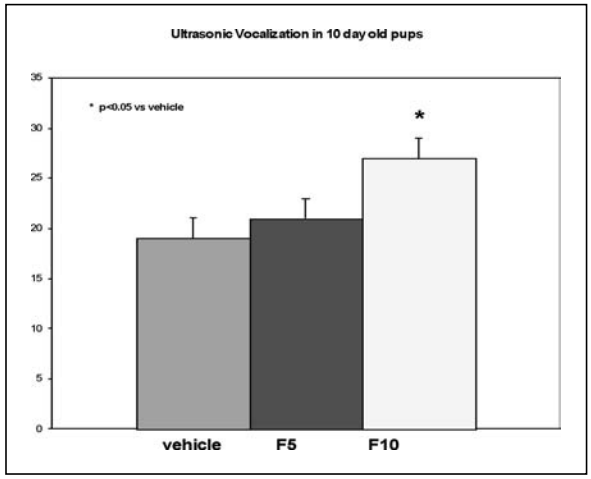

Figure 2. Ultrasonic Vocalization.

Table I. Reproduction parameters.

Groups	Females per litter (n)	Males per litter (n)	Gestation Length (days)	Dams Giving birth (%)	Pregnancy lenght (days)	Dams weight gain (%) a	Litter size at birth	Postnatal Mortality b	Male birth weight/ litter (grams)	Female birth weight/ litter (grams)	Pups dead/ born at birth
			(mean values ± S.E.M.)		*(mean values ± S.E.M.)*	*(mean values ± S.E.M.)*	*(mean values ± S.E.M.)*	*(% of pups)*	*mean (values ± S.E.M.)*	*mean (values ± S.E.M.)*	*n/n (%)*
Vehicle	4.00	4.60	21.9 ± 0.3	100.00	21.9 ± 0.31	40.84 ± 0,95	8.60 ± 0.80	0.00	6.61 ± 0.48	6.5 ± 0.45	0/00 (0%)
Fluoxetine 5 mg/Kg	5.00	4.30	21.3 ± 0.2	85.11	21.3 ± 0.27	34.20 ± 1.02	9.66 ± 0.82	0.00	5.80 ± 0.18	5.6 ± 0.20	3/87 (3.4%)
Fluoxetine 10 mg/Kg	4.20	5.00	21.4 ± 0.1	86.70	21.4 ± 0.19	34.76 ± 1.11	9.85 ± 0.65	1.03**	5.09 ± 0.21*	5.1 ± 0.23*	18/136** (31.2%)

a from day 1 to 20 of pregnancy

b from post-natal day 1 to weaning

*$p < 0.01$; **$p < 0.001$.

Motor Activity

The results of the present experiment indicate that in utero exposure to fluoxetine (5 and 10 mg/kg) did not significantly affect the number of movements in either 21- or 60-day-old male rats during a 60-min session (four 15-min blocks).

In fact, repeated measures ANOVA gave the following differences:

21st day of age:

- between treatments (F = 0.30, df = 2/24, n.s.);
- between blocks (F = 4.57, df = 3/72, $p < 0.01$);
- between treatments × blocks (F = 0.44, df = 6/72, n.s.)

60th day of age:

- between treatments (F = 1.83, df = 2/23, n.s.);
- between blocks (F = 32.61, df = 3/69, $p < 0.001$);
- between treatments × blocks (F = 0.96, df = 6/69, n.s.)

Active Avoidance Conditioning and Related Ultrasonic Vocalization

Repeated measures ANOVA for Conditioned Avoidance Responses (CAR) performed by 60-day-old male rats during a 75-trial sessions (three 25-trial blocks) gave the following differences:

(i) between groups (F = 0.93, df = 2/81, n.s.); (ii) between blocks (F = 5.61, df = 2/81, $p < 0.01$); (iii) between groups × blocks (F = 0.36, df = 4/81, n.s.)

An overall ANOVA for ultrasonic calls emitted by rats during ITI and CS-periods of the active avoidance task showed that prenatal exposure to fluoxetine did not significantly influence this behavioral parameter.

In fact the statistical analysis gave the following differences:

- ultrasonic calls emitted during the conditioned stimulus period (CS): (i) between treatments (F = 0.91, df = 2/81, n.s.); (ii) between blocks (F = 0.78, df = 2/81, n.s.); (iii) between treatments × blocks (F = 0.74, df = 4/81, n.s.);
- ultrasonic calls emitted during the intertrial interval (ITI): (i) between treatments (F = 2.03, df = 2/81, n.s.); (ii) between blocks (F = 0.19, df = 2/81, n.s.); (iii) between treatments × blocks (F = 0.19, df = 4/81, n.s.).

A one-way ANOVA for intertrial crossing performed during the active avoidance sessions showed that spontaneous activity was not affected by gestational exposure to fluoxetine at the both doses used in this study (F = 0.23, df = 2/27, n.s.) Furthermore, the results show that prenatal exposure to fluoxetine did not affect escape response latencies. One-way ANOVA gave the following differences: F = 0.80, df = 2/27, n.s.

Passive Avoidance

Prenatal treatment with fluoxetine doesn't affect the *"retention trial"* of the *"avoidance latency"* (F = 3.6; df = 2/17; n.s.); on the contrary, the *"approach latency"* of rats prenatally treated with fluoxetine 10 mg/kg resulted statistically significant ($p < 0.001$) with respect to controls. In particular, the mean of the *"approach latency values"* in the fluoxetine 10 mg/kg group resulted significantly increased with respect to the mean values of the vehicle group (F = 97; df = 2/17; $p < 0.001$) *(*Table II).

Sexual Behaviour and Related Ultrasonic Vocalization

Prenatal treatment with fluoxetine did not affect neither sexual behavior of offspring nor their related ultrasonic vocalization. In fact,

Table II. Passive avoidance task.

	Approach Latency	Avoidance Latency (24 hr after the acquisition trial)
	Acquisition trial (sec) Mean values ± S.D.	*Retention trial (sec) Mean values ± S.D*
Vehicle	10 ± 1.4	140 ± 36.0
Fluox 5 mg/kg	15 ± 3.2	180 ± 36.0
Fluox 10 mg/kg	139 ± 31.0*	125 ± 38.0

*$p < 0.001$ vs vehicle.

Table III. Statistical analysis of sexual and ultrasonic parameters.

M/IL	F = 0.02; df = 2/24; n.s.	F = 0.32; df = 2/24; n.s.	F = 0.65; df= 2/24; n.s.
M/IF	F = 0.02; df = 2/24; n.s.	F = 0.04; df = 2/24; n.s.	F = 0.40; df= 2/24; n.s.
EjL	F = 1,00; df = 2/24; n.s.	F = 0.48; df = 2/24; n.s.	F = 0.18; df= 2/24; n.s.
EjF	F = 1,00; df = 2/24; n.s.	F = 0.04; df = 2/24; n.s.	F = 0.08; df= 2/24; n.s.
PEjI	F = 1,00; df = 2/24; n.s.	F = 0.0024; df = 2/24; n.s.	F = 0.14; df= 2/24; n.s.
L50	F = 3,04; df= 2/24; n.s.	F = 0.88; df = 2/24; n.s.	F = 0.13; df= 2/24; n.s.
L22	F = 1.00; df= 2/24; n.s.	F = 0.004; df = 2/24; n.s.	F = 0.19; df= 2/24; n.s.
D22	F = 1.00; df= 2/24; n.s.	F = 0.30; df = 2/24; n.s.	F = 0.03; df= 2/24; n.s.

the statistical analysis of sexual and ultrasonic parameters gave the differences listed in the Table III.

Anatomical Malformation

Morphological evaluation at birth in newborn rats exposed to fluoxetine (10 mg/kg) during pregnancy evidenced two cases of amelia of the inferior limbs among all inspected newborns (Figure 3).

Discussion

Our results, indicating a decreased birth weight in rats prenatally treated with 10 mg/kg of fluoxetine, are in accordance with other prenatal experimental treatments with fluoxetine[37,42]. Furthermore, clinical studies on mothers prenatally exposed to SSRIs, evidenced an increased incidence of low birth weight[37,42,35] and other perinatal complications[28-30]. Our study evidenced, in rats prenatally exposed to fluoxetine 10 mg/kg, changes in some emotional parameters such as ultrasonic vocalization and passive avoidance tests. The increase in the number of ultrasonic calls/unit of time found in our study leads us to hypothesize a modification in 5-HT receptor sensitivity. Accordingly, this hypothesis could be supported by a study on the anxiety of adult rats exposed to fluoxetine (ED50 > 30 mg/kg/i.p.) in which it was shown a reduction of rat ultrasonic vocalization indicating a selective stimulation of 5-HT_{1A} and 5-HT_{2A} serotonergic receptors playing their main role on this effect[49]; a significant reduction of the hypothalamic 5-$HT_{2A/2C}$ receptor were also found in rats exposed to fluoxetine during pregnancy[37]. In accordance, a clinical study focused on the safe use of SSRIs during the last trimester of pregnancy, evidenced a neonatal withdrawal syndrome[31,50]. In addition, it was demonstrated that prenatal exposure to fluoxetine produced region-specific and age-dependent changes in 5-HT neurons and 5-HT transporters in male rat offspring[38,39]. Furthermore, in rats exposed during pregnancy to fluoxetine 10 mg/kg, we found an alteration of short-term retention memory and an increased anxiety level evidenced by an increased approach latency in the passive avoidance test. These alterations seem to be affected by prenatal exposure to fluoxetine after damage of proliferating neurons in the hippocampus[51,52]. The evidence of 2 cases of amelia in the inferior limbs found in neonatal rats prenatally exposed to fluoxetine 10 mg/kg (Figure 3) is in accordance with other studies evidencing some anatomical malformations[40].

In conclusion, the use of fluoxetine in rats during pregnancy evidenced some anatomical malformation (amelia) and an altered emotionality as functional teratogenic effect observed in different

Figure 3. Amelia of the inferior limb.

periods of the adult life. There is also evidence, in humans, that prenatal SSRIs exposure has postnatal consequences, including the neonatal adaptation[53]. Our results provide evidence that prenatal treatment with fluoxetine, at the highest dose used in the present study, produces a decrease in body weight gain, an increase in emotional reactivity (ultrasonic calls) and an alteration of motivational system (increased approach latency) of male offsprings. These results hypothesize a modification of their 5-HT receptor system with the consequent neurobehavioral outputs. Further elucidations could be drawn from parallel neuromolecular and neuromorphological studies. Due to poor number of informations available from prospective studies providing changes from decision based on non-prospective data, it was strictly recommended monitoring and interventions for patients with identified risks (e.g., poor weight gain)[34]. On the basis of our results and in accordance with previous experimental data, clinicians are strictly recommended with a prudent prescription of fluoxetine during pregnancy.

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