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In utero and lactational exposure to fluoxetine delays puberty onset in female rats offspring

Alice Hartmann dos Santos[a], Milene Leivas Vieira[a], Nathália de Azevedo Camin[a], Janete Aparecida Anselmo-Franci[b], Graziela Scalianti Ceravolo[a], Gislaine Garcia Pelosi[a], Estefânia Gastaldello Moreira[a], Ana Carolina Inhasz Kiss[c], Suzana de Fátima Paccola Mesquita[d], Daniela Cristina Ceccatto Gerardin[a,*]

[a] Department of Physiological Sciences, State University of Londrina, 86051-980 Londrina, Paraná, Brazil
[b] Department of Morphology, Estomatology and Physiology, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
[c] Department of Physiology, São Paulo State University, Botucatu, São Paulo, Brazil
[d] Department of Biology, State University of Londrina, 86051-980 Londrina, Paraná, Brazil

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ABSTRACT

Depression is one of the most prevalent disorders in the world and may occur during pregnancy and postpartum periods. Fluoxetine (FLX) has been widely prescribed for use during depression in pregnancy and lactation. This study aimed to investigate if *in utero* and lactational exposure to FLX could compromise reproductive parameters in female offspring. Wistar rats received, by daily gavage, FLX 5 mg/kg or 0.3 ml of water (control group) from the first gestational day until weaning (21 days). Assessments in the female offspring included: body weight, anogenital distance, vaginal opening, first estrus, estrous cycle, reproductive organs weight, uterine morphometric analyses, ovarian follicle and corpora lutea counting, estradiol plasmatic concentration, sexual behavior, maternal behavior and fertility test. Exposure to FLX delayed the puberty onset in female pups. The present study demonstrated that developmental exposure to FLX can deregulate the neuroendocrine hormonal control of female offspring during prepubertal and pubertal periods.

1. Introduction

Pregnancy and postpartum period are risk factors for the development or exacerbation of mental disorders, such as depression [1], due to the emergence of biological, physiological and social alterations. Approximately 40% of women that suffer from postpartum depression develop the symptoms during gestation [2]. The prevalence of perinatal depression is 10–20% [1,3].

Fluoxetine (FLX) is a selective serotonin reuptake inhibitor (SSRI) antidepressant and is the drug of choice during pregnancy due to its relative selectivity of action, efficacy, reduced side effects [4] and absence of morphological teratogenic activity [5]. FLX and its active metabolite, norfluoxetine, readily crosses the placenta in humans [6] and in experimental animals [7] and are also excreted in human milk [6]; therefore fetuses and neonates are exposed to these substances during important stages of development.

Fetal exposure to SSRI has been associated to low uterine fetal growth and symptoms related to changes in motor and somatosensory systems development [8]. Serotonin (5-HT) is known to be a trophic agent for the migration and synaptogenesis of monoaminergic neurons during brain development [9], whereas in adult life it is an important factor for neurogenesis and neuronal maintenance in the central nervous system [10]. Thus, it can be suggested that exposure to FLX during neurodevelopment may influence not only the 5-HT system, but all the monoaminergic systems. As a matter of fact, it has been reported that perinatal exposure to FLX can impair the dopaminergic (DA) system [11].

Abbreviations: 5-HT, serotonin; DA, dopamine; NA, noradrenaline; GABA, gamma-aminobutyric acid; CTR, control; FLX, fluoxetine; SSRIs, selective serotonin reuptake inhibitors; GD, gestational day; LD, lactational day; PND, postnatal day; AGD, anogenital distance; VO, vaginal opening; HPG axis, hypothalamic-pituitary-gonadal axis; GnRH, gonadotropin release hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; LM, lordosis magnitude; LQ, lordosis quotient; MB, maternal behavior; ANCOVA, analysis of covariance; ANOVA, analysis of variance; RMANOVA, repeated measures analysis of variance.

* Corresponding author at: Department of Physiological Sciences, State University of Londrina-UEL, 86051-980 Londrina, Paraná, Brazil.
E-mail addresses: dcgerardin@uel.br, danigerardin@yahoo.com.br (D.C.C. Gerardin).

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Additionally, 5-HT system may influence the reproductive function of vertebrate due to its communication with sex steroid system [12]. This interaction between the systems occurs through estrogen and progesterone receptors located in serotoninergic central neurons, demonstrating an important signaling pathway. These 5-HT central neurons can modulate several neural processes, including pituitary secretion and sexual behavior. In this way, estrogen and progesterone can interfere with these functions since these neurons are sensitive to ovarian hormones. Thus, the serotoninergic system may integrate signals from the hormonal system with the central nervous system.

Similarly to 5-HT, DA also regulates reproductive behaviors interacting with steroid hormones. Interactions between estradiol, progesterone and DA in the ventromedial nucleus of the hypothalamus regulate the lordosis reflex in rats [13]. Regarding maternal behavior (MB), the main components of the neural circuitry include the pre-optic area, the nucleus accumbens and other limbic and hypothalamic structures [14]. Despite the predominant role played by DA on MB [15], there are evidences that 5-HT is also involved in this behavior [16]. However, there are still few works evaluating the impact that developmental exposure to SSRI could have on the reproductive and central nervous systems functions. Based on these considerations, this study was carried out in order to evaluate if *in utero* and lactational exposure to FLX could disrupt the reproductive development of female offspring.

2. Material and methods

2.1. Animals and treatment

This study is part of a main study conducted in the Department of Physiological Sciences, State University of Londrina, which aimed to investigate reproductive, cardiovascular and neurological endpoints after developmental exposure to FLX. The experimental design adopted followed principles described in the guideline 426 (Developmental Neurotoxicity Studies) published by the Organization for Economic Co-Operation and Development [19] which, except for the lack of pre-mating treatment, complies with the guideline 443 (Extended One-Generation Reproductive Toxicity Study) from the same agency. All animal procedures were approved by the UEL Ethics Committee for Animal Research (16166.2012.12).

The experimental design is depicted in Fig. 1. A total of 17 male and 35 female Wistar rats (85-90 days) from the colony of the State University of Londrina (UEL) were used as parental generation. Animals were group housed five per cage in a polypropylene cage kept in a controlled environment with temperature at 21 ± 2 °C; 12 h light/dark cycle (lights on at 6:00 a.m.) and had free access to regular lab chow (Nuvital™, Paraná, Brazil) and tap water bottles. Autoclaved wood shaver bedding was used to prevent contamination from mycoestrogens in corncob bedding. Female and male rats were maintained separated by sex during 15 days prior to the beginning of mating. Rats were mated (2 females and 1 male per cage) and gestational day (GD) 0 was determined if there were sperm and estrus phase cells in vaginal smears. Dams were randomly divided into 2 groups:

- Control (CTR): 17 dams received tap water daily, by gavage, from GD 0 to postnatal day (PND) 21;
- FLX: 18 dams received FLX (5 mg/kg, Daforin™ oral solution, EMS Laboratory, Brazil), by gavage, from GD 0 to PND 21.

Dams were daily treated at 11:00-13:00 p.m. and doses were adjusted each 3 days according to weight.

In humans, FLX prescription ranges from 20 to 80 mg/day, which would correspond to approximately 0.29–1.14 mg/kg. Considering that the precautionary principle considers animals more resistant than humans, higher doses are tested in animals. Preliminary studies of our group showed that the dose of 10 mg/kg decreased the number of pups per litter (data not shown). Vorhees et al. reported that prenatal exposure to 12 mg/kg FLX resulted in a significant increase in offspring mortality from birth to PND 7 with no alterations observed in pups exposed to 5 mg/kg of FLX [17]. Bairy et al. demonstrated that prenatal exposure to 12 mg/kg FLX resulted in a significant reduction in birth weight and that this alteration was not observed at 8 mg/kg dose [18]. In this way, the dose of 5 mg/kg was chosen to ensure no influence on litter size and weight.

At birth (PND 0), pups were counted, the sex determined and the litters were weighed. On PND 4, litters were culled to 10 pups keeping 5 males and 5 females whenever possible. From each litter, 1–2 female pups were used for this study. The anogenital distance, body weight and vaginal opening were observed in the 2 female pups per litter, whenever possible. After that, one female was allocated to the behavioral evaluation and the other to the assessment of non-behavioral reproductive parameters.

2.2. Parameters analyzed in female offspring during development (PND 0–60)

2.2.1. Body weight

Female pups body weight was measured on PND 0, 7, 14 and 21 (CTR: 17 litters and FLX: 18 litters). This data is expressed as litter mean.

2.2.2. Physical sexual development

The anogenital distance (AGD, distance from the anus to the genital tubercle) was measured on PND 0 and 21 (CTR: 17 litters and FLX: 18 litters). AGD was normalized through its division by the cube root of body weight. From PND 30 on, females from both groups were daily evaluated for vaginal opening (VO, CTR: 27 litters and FLX: 28 litters) in order to determine the day in which complete VO occurred. In this day, females were weighed. Starting from the day of VO, daily vaginal smears were collected to detect the day of the first estrus (CTR: 11 litters and FLX: 11 litters), characterized by the predominance of cornified epithelial cells [20]. These parameters were evaluated in 2 littermates and data are expressed as litter means.

2.3. Parameters analyzed in female offspring during adulthood

2.3.1. Estrous cycle evaluation

On PND 75, the estrous cyclicity of female rats (CTR: 12 rats and FLX: 11 rats) was assessed through vaginal smears collected over a period of 15 days as previously described in Ref. [21]. The cycle phases were cytologically determined by the following characteristics: predominance of nucleated epithelial cells (proestrus); predominance of cornified epithelial cells (estrus); presence of cornified and nucleated epithelial cells and leukocytes (metaestrus); predominance of leukocytes (diestrus). The total frequency of each phase was used to calculate the total length (in days) of the proestrus, estrus, metaestrus and diestrus and the estrous cycle length.

2.3.2. Collection of tissues and organs

After the estrous cycle evaluation and during an estrus phase (PND 90–95), females (n = 10/group) were weighed, deeply anesthetized with thiopental and laparatomized. Blood samples were collected from abdominal aorta for quantification of plasmatic estradiol. The ovaries and uteri (with fluid) were collected and weighed. After that, they were euthanized by decapitation. The right ovary and the medial portion of the right uterine horn were fixed in Bouin, dehydrated in ethanol and embedded in Paraplast®