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Reproductive parameters of female Wistar rats treated with methylphenidate during development

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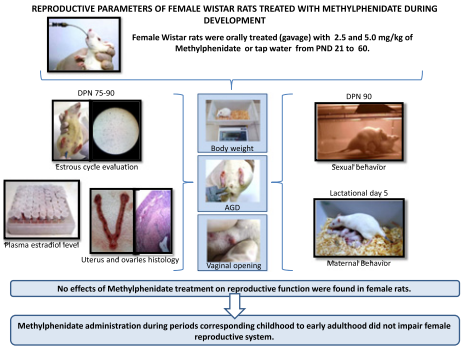
HIGHLIGHTS

- Female rats were treated with MPH from late infancy to early adulthood periods.
- MPH treatment does not affect reproductive function during development.
- No effect was detected in sexual parameters in adult female rats.

GRAPHICAL ABSTRACT



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ABSTRACT

Methylphenidate (MPH), a psychoactive agent that acts mainly by blocking the uptake of dopamine, is the main drug used to treat Attention Deficit Hyperactivity Disorder in children and adolescents. During development, important changes in brain architecture and plasticity occur, these changes, sensitive to exposure to stimulant drugs, are important in the control of GnRH secretion, influencing the release of sex hormones throughout the ovarian cycle. This study investigated the effects of repeated treatment with MPH during development on reproductive parameters of adult female rats. Wistar rats received MPH 2.5 mg/kg, MPH 5.0 mg/kg, or tap water (gavage) from postnatal day (PND) 21 to PND 60. From PND 75, one subgroup of females was selected for evaluation of estrous cycle, estradiol levels, weight of sexual organs, and histomorphological analysis of ovary follicles and uterus. In another subgroup, the sexual and maternal behaviors were evaluated at PND 90 and on lactational day 5, respectively. No significant alterations were observed in the MPH groups. This study demonstrated that repeated administration of MPH during the period corresponding to childhood to early adulthood does not interfere in the reproductive function of female rats in adulthood.

Abbreviations: ADHD, attention deficit hyperactivity disorder; MPH, methylphenidate; PND, post-natal day; AGD, anogenital distance; LD, lactational day.

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1. Introduction

Over the past few decades, methylphenidate (MPH) has become the main psychostimulant drug prescribed to children and adolescents in the treatment of attention deficit hyperactivity disorder (ADHD) [1–4]. This neurodevelopment disorder is characterized by persistent symptoms of inattention, hyperactivity and impulsivity [5], with a worldwide prevalence of 3.4% in children and adolescents [6].

The therapeutic effect of MPH is mainly due to increasing dopamine (DA) signaling, primarily by blockade of the DA reuptake transporter [7,8], although noradrenergic receptors may also contribute to MPH activity [9].

Several studies have shown that the prevalence of ADHD is higher in boys than in girls [10,11]. However, longitudinal studies in school-aged children have indicated an increase in the proportion of girls using MPH [1,2,12], which raises major concerns for public health due to possible persistent neurobehavioral changes [13–15].

In mammalians, important changes in the brain architecture and plasticity occur in the period from immediately before birth until adolescence and these changes could be influenced by exposure to psychotropic stimulant drugs [16–18]. Monoamines, such as dopamine (DA) and noradrenalin (NA) are widely distributed in the brain and have a strong role in the neuroendocrine control of GnRH release in these periods [19–21]. Alterations in GnRH secretion modulated by DA and NA neurons could change the LH secretion profile throughout the ovarian cycle, including the pre-ovulatory LH surge [22–24]. The presence of DA receptors in the ovaries of rodents also suggests that the function of the corpus luteum and/or follicular development may be regulated by catecholaminergic innervation during the estrous cycle [25–27]. In addition, interactions between central monoaminergic systems and steroid hormones in the integration of reproductive behavior are widely reported in the literature [20,21].

In this sense, despite the efficacy of MPH to reduce ADHD symptoms [28,29], little is known about the long-term changes that treatment with MPH could induce in the developing organism, including the reproductive function. Previously published data from our laboratory show that MPH administration during late infancy until early adulthood may impact on the reproductive function of adult male rats [30]. At the same time, there is a lack of studies evaluating the effect of early treatment with MPH and its effects on the reproduction of adult female rats.

Based on these considerations, this study was conducted to evaluate the long-lasting effects on reproductive function in adult female rats submitted to MPH treatment similar to that established for ADHD, from the late infancy period to early adulthood [31].

2. Materials and methods

2.1. Animals and treatment

A total of 10 male and 20 female Wistar rats (85–90 days) from the colony of the State University of Londrina (UEL) were used as parental generation. The animals were kept in a controlled environment at a temperature of 21 ± 2 °C; 12 h light/dark cycle (lights on at 6:00 a.m.) with free access to regular lab chow (Nuvital™, Paraná, Brazil) and water. The rats were mated (2 females and 1 male per cage) and gestational day 0 was determined if there were sperm and estrous phase cells in vaginal smears. On post-natal day (PND) 4, litters were culled to 8 pups keeping 4 males and 4 females wherever possible. Female pups were weaned on PND 21 and divided into three groups.

- Control group (CTR): animals were gavaged with tap water daily, from PND 21 to PND 60 ($n = 28$);
- MPH 2.5 mg group (MPH 2.5): animals were gavaged with 2.5 mg/kg of MPH (Ritalin™, Novartis) daily, from PND 21 to PND 60 ($n = 20$) [30];
- MPH 5.0 mg group (MPH 5.0): animals were gavaged with 5.0 mg/kg of MPH (Ritalin™, Novartis) daily, from PND 21 to PND 60 ($n = 22$) [30].

To avoid sibling effects, no littermates were used for the same group. Rats were treated daily at 4–6 p.m. The drug was dissolved in tap water immediately prior to the treatment.

In rodents, the puberty period corresponds to the age window between postnatal days 28 (female)/35 (male) and 50 [31]. At puberty, the hypothalamus receives neuronal input from many brain centers for GnRH release, these changes are particularly important in females, maintaining the regularity of the estrous cycle [32], in addition to structuring relevant hypothalamic areas for reproductive behaviors [33]. In this way, the females were treated from PND 21 to PND 60, since the treatment with MPH in humans, although beginning during childhood, can persist into adulthood [34], and in order to cover the critical developmental stages of the reproductive systems.

The effective dose range in children is 0.3–1.0 mg/kg MPH [35]. Applying the $BW^{3/4}$ scaling [36], the equivalent dose in rats would be 1.7–5.5 mg/kg. The highest dose used in this study (5.0 mg/kg) would be equivalent to a clinically relevant dose in humans and higher doses were not tested since it is already described in the literature that oral administration of 5.0 mg/kg does not compromise weight gain [37]. Methylphenidate chloride is expressed as the formula weight for Ritalin™ (10 mg, a 50:50 racemic mixture of the D-threo-and L-threo-methylphenidate enantiomers). The oral gavage method was chosen in order to provide the same administration route used in humans.

All animal procedures were approved by the UEL Ethics Committee for Animal Research (CEUA 16381.2012.45). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments. The experimental protocol is diagramed in Fig. 1.

2.2. Parameters analyzed during development (PND 21–60)

2.2.1. Body weight

Body weight was measured daily during the treatment period as well as observation of signs of toxicity (e.g. lacrimation, piloerection, unusual respiratory pattern and tremors).

2.2.2. Physical sexual development

The anogenital distance (AGD, distance from the anus to the genital tubercle) was measured using a vernier caliper on PND 21, 28 and 35. The measurements obtained were normalized through their division by the cube root of bodyweight [38]. From PND 30, the vaginal opening was verified daily and considered as an indicator of the onset of sexual maturity.

2.3. Parameters analyzed in adulthood (from PND 75)

For the evaluation of female reproductive development, each group (CTR, MPH 2.5 and MPH 5.0) was divided into 2 subgroups ($n = 8$–14/subgroup): one group for estrous cyclicity, estradiol level, sexual organ weight and histomorphological analysis of the ovary follicles and uterus and the other for sexual and maternal behavior evaluation.

2.3.1. Estrous cycle evaluation

The normal length of the estrous cycle of the rodent strain used in this study ranges from 4 to 5 days [39]. Beginning at PND 75, vaginal smears were obtained daily, always at the same time in the morning, over a period of 15 days. The material was observed under a light microscope and the estrous cycle phases were classified as proestrus, estrus, metaestrus and diestrus. Proestrus was defined by smears possessing the prevalence of nucleated epithelial cells and no leucocytes. Estrus

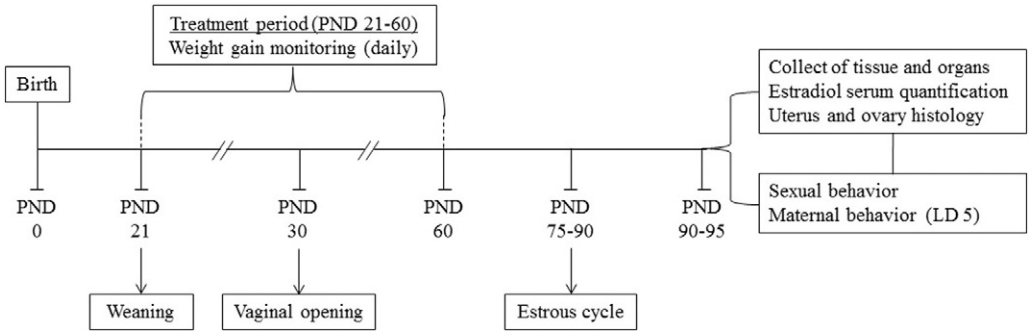

Fig. 1. Diagram of the experimental design. PND: postnatal day.

was defined as smears with a large number of cornified epithelial cells, some nucleated cells and the absence of leucocytes. Metaestrus and diestrus smears were defined by the presence of leucocytes. Coefficients of proestrus (Cp), estrus (Ce) and metaestrus/diestrus (Cmd) were determined by the formula $C = a / b \cdot 100\%$ [40], where C is the coefficient of the cycle period, a is the number of days of the corresponding cycle period in the course of the observation, and b is the total duration of the complete cycles (in days). The estrous cycle duration was calculated as the number of days between one estrous phase to the next.

2.3.2. Plasmatic estradiol quantification

After evaluation of the estrous cycle (PND 90) and during the estrus phase, the female rats were weighed, euthanized with sodium thiopental (40 mg/kg), and blood samples were collected from the abdominal aorta into syringes containing heparin, always at the same time. Blood samples were centrifuged (2500 rpm for 20 min at 2 °C) and the plasma frozen until assayed. Blood plasma estradiol was measured by radioimmunoassay using 17-β Estradiol (E2) Double Antibody RIA Kit (MP Biomedicals, Orangeburg, NY) according to the manufacturer's instructions. Samples were analyzed in a double assay format, the minimum sensitivity of the assay was 1.2 pg/mL and intra-assay and inter-assay coefficients of variation were 2.5% and 7.0%, respectively.

2.3.3. Collection of tissue and organs

The ovaries and uterus (with fluid) were removed and their weights (absolute and relative to body weight) determined. Organs (left ovary and uterus) were fixed in Bouin's solution for 24 h before being stored in ethanol 70° for histomorphological analysis.

2.3.4. Analysis of reproductive organs

The preserved organs were cut into tissue fragments, dehydrated in increasing concentrations of ethanol, and embedded in paraffin. Blocks were sectioned at 5 μm (6 sections per animal/group) separated by a distance of 50 μm, stained with hematoxylin and eosin (HE) and analyzed under a phase-contrast microscope. The numbers of each type of follicle and formed corpora lutea were counted in unilateral ovaries. Follicles were assessed by light microscopy (400× magnification). Primordial (types 1–3b), growing (types 4–5b), and antral (types 6–8) follicles were identified using a slight modification of Pedersen and Peters criteria [41]. The mean number of ovarian follicles and corpora lutea were calculated in the selected sections. In the uterus, the endometrial height was measured, in three sections per animal, using a linear reticule micrometer (OSM-223287, Olympus), coupled to an ocular microscope at 100× magnification. Uterine epithelium, endometrial stroma, myometrium, and perimetrium were evaluated in the middle portion of the uterus, relative to the position of the oviduct and ovary. The endometrial glands and blood vessels were not measured. In each section, five different regions were analyzed, resulting in a total of 15 measurements per animal.

2.3.5. Sexual behavior evaluation

The animals were allowed a 15-day period of adaptation to the reversed light/dark cycle before the beginning of the evaluations. The female sexual behavior (starting on PND 90) was recorded by a video camera, linked to a monitor in an adjacent room with sexually experienced males, under dim red light, 3 to 4 h after a proestrus smear was observed [42]. Initially, the male was inserted into the observation cage for 10 min and then the female was placed in the same cage. The test lasted until ten mounts had been observed [43] and results were expressed as the lordosis quotient ($LQ = [number\ of\ lordosis\ /\ total\ mounts] \times 100$) [44]. The frequency of each lordosis magnitude, on a scale of 0 to 3, was ranked as follows: 0) absence of lordosis; 1) the female showed little flex of spine, head and hips slightly elevated from floor; 2) the female showed spinal flex and head raise close to an angle of 30° with the floor; 3) maximum lordosis, with accented spinal flex and the head inclined at an angle of 45° or more relative to the floor. Mean lordosis scores (LS = total number of lordosis points / total number of lordosis responses) were assigned for each test [45]. All females were used only once.

2.3.6. Maternal behavior

Evaluation of maternal behavior was carried out between 8:00 a.m. and 2:00 p.m. on lactational day (LD) 5, since at an early time point the energy cost of lactation is lower and the pups have limited motor capacity. On the test day, all pups were removed from the home cage and the nest was destroyed. After 30 min, the pups were returned to the cage and mother–pup interaction was recorded for 30 min. Latency for retrieval behavior and total time grouping, pup grooming, off pups (defined as the amount of time the rat spent without any kind of interaction with the pups regardless of her position in the cage), and nest building were observed. All behavioral analyses were performed using Etholog software [46].

2.4. Statistical analysis

An exploratory analysis was conducted to evaluate normal distribution (Shapiro-Wilk test) and homogeneity of variance (Levene's test) of each variable. Variables that presented normal distribution and homogeneity of variance were analyzed by ANOVA complemented with the Bonferroni post-hoc test, with data being presented as mean ± standard error of the mean (SEM). Conversely, for other variables the Kruskal-Wallis H complemented with Dunn's test were performed with the presentation of data as median (1st and 3rd quartiles). Analysis of co-variance (ANCOVA) using the body weight was used to detect the effect of each treatment on vaginal opening. Differences were considered significant if $p < 0.05$. All statistical analyses were performed using SPSS (IBM, SPSS Statistics v19).

3. Results

3.1. Parameters analyzed during development (PND 21–60)

3.1.1. Body weight and physical sexual development

The repeated administration of MPH did not affect body weight gain during MPH treatment (CTR group: 152.42 ± 3.37 / MPH 2.5 group: 152.56 ± 4.58 / MPH 5.0 group: 150.97 ± 3.37) [$F_{(2,69)} = 0.05$, $p =$ 0,95], relative AGD (mm/$g^{1/3}$) (data not shown), or the day of vaginal opening (with the body weight on the day of the vaginal opening used as a covariant) [$F_{(2,66)} = 0.31$, $p = 0.73$]: CTR group = 36.04 ± 0.57, $n = 28$; MPH 2.5 group = 35.35 ± 0.66, $n = 20$; MPH 5.0 group = 34.73 ± 0.60, $n = 22$. The covariate body weight was significantly related to vaginal opening [$F_{(2,66)} = 36.05$, $p < 0.01$].

3.2. Parameters analyzed in adulthood

3.2.1. Estrous cyclicity (DPN 75–90)

Estrous data collected between PND 75–90 were subjected to statistical analysis and are presented in Table 1. A non-parametric Kruskal-Wallis H test showed no significant differences in the coefficients of estrus, χ^2 (2) = 1.32, $p = 0.52$, metaestrus/diestrus, χ^2 (2) = 2.32, $p = 0.31$, and proestrus, χ^2 (2) = 0.00, $p = 0.99$, or the estrous cycle length, χ^2 (2) = 1.34, $p = 0.51$ (CTR group, $n = 14$ / MPH 2.5 group, $n = 10$ / MPH 5.0 group, $n = 10$).

3.2.2. Plasma estradiol quantification

The repeated administration of MPH did not cause significant alteration in the plasma estradiol level in adulthood (CTR group: 174.23 ± 34.34 / MPH 2.5 group: 227.06 ± 52.00 / MPH 5.0 group: 179.59 ± 32.64; pg/ml) [$F_{(2,29)} = 0.51$, $p = 0.61$] ($n = 10$/group, since that 14 samples were collected in the CTR group, only 10 were randomly chosen, and this number is usually enough for proper statistical analysis).

3.2.3. Body weight and reproductive organ weight

The final body weight and weight of reproductive organs of the adult females are presented in Table 2. No significant differences as a result of the MPH treatment were found in the final body weight [$F_{(2,33)} = 0.65$, $p = 0.53$]. Mean weights of the uterus and ovaries (absolute and relative to body weight) were similar between the experimental groups (CTR group, $n = 14$ / MPH 2.5 group, $n = 10$ / MPH 5.0 group, $n = 10$).

3.2.4. Ovarian follicle quantification and morphometric measurements

There were no significant differences (CTR group, $n = 12$ / MPH 2.5 group, $n = 9$ / MPH 5.0 group, $n = 8$, $p > 0.05$, the number of samples were reduced in all groups once this number is usually enough for proper statistical analysis) in the corpora lutea [$F_{(2,28)} = 1.46$, $p = 0.25$] and follicles counts (primary, grow and antral) among the experimental groups (Table 3) observed 40 days after the treatment period. There was no evidence of morphological impairment in the perimetrium [$F_{(2,28)} = 0.61$, $p = 0.55$], myometrium [$F_{(2,28)} = 1.15$, $p = 0.33$] or in the luminal epithelium [$F_{(2,28)} = 0.03$, $p = 0.97$] of MPH-treated rats, compared to the control groups. In MPH 2.5 animals, the endometrial stroma [$F_{(2,28)} = 0.41$, $p = 0.05$] was increased compared to MPH 5.0 group.

Table 2
Body weight and wet weight of organs of adult female rats.

	CTR [14]	MPH 2.5 [10]	MPH 5.0 [10]
Final body weight (g)	252.90 ± 5.33	248.53 ± 6.44	243.49 ± 6.24
Absolute weights (mg)			
Uterus	446.21 ± 21.63	432.50 ± 15.48	428.10 ± 14.92
Ovary (right)	40.85 ± 2.41	42.10 ± 2.47	45.30 ± 2.62
Ovary (left)	38.86 ± 2.48	38.10 ± 2.41	37.60 ± 2.15
Relative weights (mg/100 g)			
Uterus	176.99 ± 8.65	175.04 ± 7.62	176.42 ± 6.55
Ovary (right)	16.24 ± 1.00	16.99 ± 1.00	18.69 ± 1.18
Ovary (left)	15.39 ± 0.94	15.41 ± 1.03	15.45 ± 0.83

Data are means ± SEM. Numbers in brackets represent the number of animals/group. ANOVA, $p > 0.05$. CTR: tap water; MPH 2.5: Methylphenidate 2.5 mg/kg; MPH 5.0: Methylphenidate 5.0 mg/kg.

3.2.5. Sexual behavior

Regarding the sexual behavior, all mounts were accompanied by lordosis (LQ = 100%) in all experimental groups. Moreover, treatment with MPH did not alter the lordosis score (CTR group: 2.95 (2.83–3.00), $n = 14$ / MPH 2.5 group: 2.90 (2.80–3.00), $n =$ 10 / MPH 5.0 group: 3.00 (2.80–3.00), $n = 12$) as shown by the Kruskal-Wallis H test, χ^2 (2) = 0.20, $p = 0.91$.

3.2.6. Maternal behavior

Maternal behavior results (LD 5) are shown in Table 4. Two CTR and three MPH 5.0 rats did not retrieve all pups. Data from these animals were removed from the analysis. There was no significant difference (Fisher's test, $p > 0.05$) in the percentage of MPH-treated groups that displayed retrieving behavior compared to the CTR group. Moreover, the repeated administration of MPH did not cause significant alterations in retrieval behavior, time spent grouping, pup grooming, off pups or nest building (ANOVA, $p > 0.05$).

4. Discussion

The MPH dosing regimen used in this study was chosen to mimic, in humans, the clinically therapeutic doses and administration route. In humans, the main MPH urinary metabolite is the deesterified product, ritalinic acid, accounting for 80% of the dose [47,48]. Similar to humans, a major metabolite in rat urine is ritalinic acid [49]. In rodents, pharmacokinetics parameters have been determined by a number of studies [50,51]. In adult male CD-1 mice, the plasma concentration of MPH was measured at two end points (15 and 30 min) after a single oral administration at a dose of 5 mg/kg, values exceeded (above 60 ng/mL) the typical therapeutic range of MPH [50], which is between 8 and 10 ng/mL in children (about 80% of DA transporters occupancy) [52]. In a study conducted in adult Sprague-Dawley rats, it was observed that MPH at a dose of 5 mg/kg has its maximum effect, increasing extracellular DA concentration in the nucleus accumbens, 40 min after the oral administration and returns to baseline values in approximately 2.5 h [51]. Based on these considerations, it can be stated that in the

Table 1
Coefficients of the different stages of estrous cycle and estrous cycle length (PND 75–90).

	CTR [14]	MPH 2.5 [10]	MPH 5.0 [10]
Coefficient of proestrus (Cp)	20.00 (20.00–25.00)	20.00 (20.00–26.67)	20.00 (20.00–20.67)
Coefficient of estrus (Ce)	26.67 (21.67–26.67)	26.67 (26.67–31.67)	26.67 (26.67–28.67)
Coefficient of metaestrus/diestrus (Cmd)	53.33 (53.33–53.33)	46.67 (46.67–53.33)	53.33 (48.33–53.33)
Estrus cycle length (days)	4.82 (4.06–5.00)	5.00 (3.75–5.00)	5.00 (5.00–5.00)

Data are medians (1st and 3rd quartiles). Numbers in brackets represent the number of animals/group. Kruskal–Wallis H, $p > 0.05$. Cp, Ce, and Cmd were determined by the formula $C = a / b \cdot 100\%$, where C is the coefficient of the cycle period, a is the number of days of the corresponding cycle period in the course of the observation, and b is the total duration of the complete cycles (in days). CTR: tap water; MPH 2.5: Methylphenidate 2.5 mg/kg; MPH 5.0: Methylphenidate 5.0 mg/kg.

Table 3
Corpora lutea count, ovarian follicle quantification and morphometric analysis of uterus.

	CTR [12]	MPH 2.5 [9]	MPH 5.0 [8]
Corpora lutea	6.44 ± 0.50	7.76 ± 0.89	6.27 ± 0.56
Follicles			
Primary	7.76 ± 1.31	6.74 ± 1.09	10.72 ± 1.23
Grow	4.16 ± 0.42	4.59 ± 0.75	5.23 ± 0.88
Antral	8.08 ± 0.31	9.29 ± 1.10	8.58 ± 1.05
Uterus parameters (μm)			
Perimetrium	14.12 ± 0.87	13.36 ± 0.35	13.01 ± 0.75
Endometrial stroma	651.97 ± 32.84	723.51 ± 33.25[#]	581.09 ± 43.54
Myometrium	418.95 ± 28.28	432.30 ± 34.56	365.77 ± 28.46
Luminal epithelium	27.25 ± 1.12	26.98 ± 0.95	26.86 ± 1.56

Data are means ± SEM. Numbers in brackets represent the number of animals/group. CTR: tap water; MPH 2.5: Methylphenidate 2.5 mg/kg; MPH 5.0: Methylphenidate 5.0 mg/kg.
[#] $p < 0.05$ compared to MPH 5.0 group (ANOVA complemented with Bonferroni).

present study, the doses used for MPH were able to interfere with DA transporters.

Data in the literature shows that children treated with MPH can present either weight loss [53] or the absence of this effect [54]. In the present study, the repeated administration of MPH did not affect body weight of female rats during the treatment period. In developing Wistar rats (PND 7–70), oral treatment with MPH at a dose of 50 mg/kg/day decreased the body weight of females only during the pre-pubertal period (days 16, 17, and 21 to 28) while at a dose of 100 mg/kg/day, the decrease in weight gain is observed from day 12 and persists throughout the treatment [37]. Similar to our data, females that received 5 mg/kg/day presented weight gain similar to the controls [37]. Additionally, as already published in other studies, at a daily dose up to 5 mg/kg, repeated oral treatment with MPH during development did not affect weight gain [55,56]. Since changes in body weight gain could impair sexual maturation [57] and hormonal profile [58], MPH doses that have no influence on body weight were selected for the present study [30].

There is increasing concern regarding exposure to xenobiotics during critical periods, such as juvenile and puberty periods, and the possibility of decreasing sensitivity to sex hormones [59]. Changes in hormonal profile during this period could produce permanent changes in brain function and consequently in behavior [60]. In mammalians, puberty is triggered by the activation of the gonadotropin-releasing hormone (GnRH) neurons from a state of relative quiescence, activating the hypothalamic-pituitary-gonadal (HPG) axis, beginning the sequence of pubertal development [61–63]. Hypothalamus is controlled by numerous afferent inputs from many neuronal systems with stimulatory and inhibitory effects on GnRH release [64]. Catecholamines are present in all areas of migration in GnRH neurons during prenatal development [19] and it has also been demonstrated in vitro that approximately 50% of GnRH neurons express D1-and/or D2-like receptors with a strong suppressive role from dopaminergic inputs [23], which may suggest a major influence of DA in this system. In this study, despite the potential interference of DA agonists on sexual development, repeated treatment with MPH did not affect the onset of puberty, observed by the day of vaginal opening.

The estrous cycle is controlled by a cascade of neuroendocrine events, involving the activation of the hypothalamus-pituitary-gonadal axis. Although changes in puberty and the estrous cycle were not observed in the present study, in Sprague-Dawley rats, 4 weeks of daily subcutaneous administrations of MPH (450 μg, beginning on PND 28) impaired ovarian folliculogenesis and retarded pituitary LH release and the occurrence of vaginal opening [32]. Moreover, it seems that earlier MPH treatment could be directly related with long lasting impairment of female reproductive function in Sprague-Dawley rats, since a persistent decrease in the number of regular estrous cycles was observed when the MPH treatment started on PND 5–7 (35 mg/kg/day, subcutaneously), however when the injections were initiated on PND 21–23, impairment of the estrous cycle was observed only during the treatment period (30 days) without persistence during the post-treatment observation period (18 days) [65]. In our study, in order to assess whether early treatment with MPH could interfere with reproductive function of adult female rats, the monitoring of the estrous cycle was performed approximately two weeks after the end of MPH administrations. The different results in the literature compared to our study could be related to the different administration routes, treatment period, window of post-treatment observation and decrease in body weight observed in Chatterjee-Chakrabarty et al. [32] and Greeley and Kizer [65], besides using different strains of rats.

Our study aimed to evaluate the MPH effects around 40 days after the end of treatment, since MPH is commonly prescribed for children and adolescents with ADHD and considering the possibility of persistent symptoms on reproductive function in adulthood. Organ weight changes are often associated with treatment-related toxic effects of a drug [66], as in hormone dependent organs in animals treated with drugs that act on the HPG axis [67]. Although MPH could promote changes in the sex hormone profile due to interference in the pulsatile release of hypothalamic GnRH [32], in our study, the weight of the reproductive organs of adult animals was not changed after the treatment with MPH from childhood to young adulthood. Previously published data from our laboratory demonstrated that MPH administration (PND 18–20) is unable to change the uterine weight in immature female rats (PND 21), at least at the tested doses (2.5 and 5.0 mg/kg, daily) [68]. In adult females, one study demonstrated a transient increase in the weight of the ovaries after 90 days of a treatment-regimen with D-MPH active enantiomer, but only at doses of 50 mg/kg/day [69]. Although the lack of studies investigating the effects of MPH on the weight of the female reproductive organs restricts the discussion, our results suggest that early treatment (PND 21–60) with MPH at doses of 2.5 and 5.0 mg/kg does not cause changes in female reproductive organ weights at the time of euthanasia.

It was noticed that even though MPH may alter important hormonal systems for female reproductive function, it does not appear to be able to interfere in circulating estradiol concentrations. In this study, repeated treatment with MPH during the development of female rats did not change the plasma concentration of this hormone. Similar results were also found in Chatterjee-Chakrabarty et al. [32], where the values for serum estradiol levels in animals subcutaneously treated with MPH (450 μg/day, PND 28–56) were similar to the control group, and in Ferguson and Boctor [70], in which oral treatment with MPH (3 mg/day) during PND 29–50, did not implicate in changes in estradiol plasmatic levels in adult females (PND 90).

In the rodent uterus, morphometric measurement of the endometrial epithelium is directly correlated with estradiol levels and based on the stage of the cycle, uterine thickness at estrus is significantly greater compared to other stages [71,72]. No influence on the uterus measurement (perimetrium, endometrial stroma, myometrium and luminal epithelium) of females in the estrus phase was observed in this study, as observed in the follicle count. These findings were expected, since estradiol levels were unchanged at the time of euthanasia.

Table 4
Maternal behavior of adult female rats at LD 5.

Parameters (s)	CTR [12]	MPH 2.5 [10]	MPH 5.0 [9]
Time to retrieve first pup	143.07 ± 55.19	46.40 ± 8.35	84.10 ± 42.01
Time to retrieve 4 pups	356.49 ± 67.64	237.42 ± 60.47	239.39 ± 47.78
Time to retrieve all pups	450.06 ± 75.05	333.03 ± 51.79	360.81 ± 84.96
Total time grouping	118.27 ± 21.11	105.24 ± 16.12	159.51 ± 34.35
Pup grooming	384.58 ± 36.69	379.97 ± 67.61	334.13 ± 73.55
Nest building	147.14 ± 24.78	219.48 ± 54.34	206.46 ± 41.19
Off pups	1102.68 ± 22.97	1075.09 ± 93.25	1081.42 ± 36.75

Data are means ± SEM. Numbers in brackets represent the number of animals/group. ANOVA, $p > 0.05$. CTR: tap water; MPH 2.5: Methylphenidate 2.5 mg/kg; MPH 5.0: Methylphenidate 5.0 mg/kg.

It is proposed that projections from the hypothalamic medial preoptic area interact with the mesolimbic DA system to promote appetitive and consummatory aspects of sexual and maternal behavior [73]. Despite the MPH capacity to change DA levels during development of the organism and considering the DA system involvement in the establishment of reproductive behavior, no influence on sexual behavior was observed following the MPH treatment in the present study. Our study, indeed, is the first to investigate the effects of repeated treatment with MPH on sexual behavior of female rats.

The maternal behavior evaluation, in turn, was performed on females at LD 5, about eight weeks after the end of treatment with MPH, without changes in observed parameters. Two studies have evaluated the effects of MPH on maternal behavior in mice. In virgin female CD1 mice, the subcutaneous administration of MPH (5 mg/kg), starting 3 days before pup exposure and for a 10 day test period, reduced pup licking and crouch over pups [74]. In another study, in Balb-c mice, oral MPH administration during early lactation (5 mg/kg, days 2 to 4) impaired maternal behavior at LD 5, increasing the latency to retrieve the pups [75]. Compared to both studies, the absence of alterations in maternal behavior in our study could be related to differences in the experimental protocol and the time proximity between the MPH administration and behavior analysis, in addition to using Wistar rats. It is known that drugs that act similarly to MPH, such as cocaine and methamphetamine can affect maternal behavior motivation [76–78]. However, it is difficult to compare the results, since these psychostimulants differ in affinity to the carriers, as well as effects by other monoamines [79,80], i.e., even though DA is the major neurotransmitter involved in the reward and motivation of maternal behavior [81,82], NA and serotonin may also modulate this behavior [83,84]. Although there is evidence suggesting the development of central nervous system plasticity changes after MPH treatment [75], the lack of MPH effect on maternal behavior in our study could suggest that, probably, the potential DA neurotransmitter changes induced by MPH were no longer present at the time of behavior evaluation. In the case that changes in brain plasticity that may affect maternal behavior happened, they did not persist in adult animals.

5. Conclusions

The present study demonstrated that repeated MPH administration during the period corresponding to childhood to early adulthood did not impair the female reproductive system.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

[1] E. Romano, B. RH, W. HX, P. Robaey, Tremblay RE. Prevalence of methylphenidate use and change over a two-year period: a nationwide study of 2- to 11-year-old Canadian children, J. Pediatr. 141 (2002) 71–75.
[2] C. Gumy, T. Huissoud, F. Dubois-Arber, Prevalence of methylphenidate prescription among school-aged children in a Swiss population: increase in the number of prescriptions in the Swiss Canton of Vaud, from 2002 to 2005, and changes in patient demographics, J. Atten. Disord. 14 (2010) 267–272.
[3] E. Perini, D.R.G. Junqueira, L.G.C. Lana, T.C.B. Luz, Prescription, dispensation and marketing patterns of methylphenidate, Rev. Saúde Pública 48 (2014) 873–880.
[4] M.A. Aboul-ata, A.A. Amin, The prevalence of ADHD in Fayoum City (Egypt) among school-age children: depending on a DSM-5-based rating scale, J. Atten. Disord. (2015) [Epub ahead of print].
[5] American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR), 4th ed. text revision American Psychiatric Association, Washington, DC, 2000.
[6] G.V. Polanczyk, G.A. Salum, C.A. SugayaLS, L.A. Rohde, Annual research review: a meta-analysis of the worldwide prevalence of mental disorders in children and adolescents, J. Child Psychol. Psychiatry 56 (2015) 345–365.
[7] T.E. Wilens, Effects of methylphenidate on the catecholaminergic system in attention-deficit/hyperactivity disorder, J. Clin. Psychopharmacol. 28 (2008) S46–S53.
[8] N.D. Volkow, J.S. Fowler, G.J. Wang, Y.S. Ding, S.J. Gatley, Role of dopamine in the therapeutic and reinforcing effects of methylphenidate in humans: results from imaging studies, Eur. Neuropsychopharmacol. 12 (2002) 557–566.
[9] R. Kuczenski, D.S. Segal, Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine, J. Neurochem. 68 (1997) 2032–2037.
[10] P. Pastor, C. Reuben, C. Duran, L. Hawkins, Association between diagnosed ADHD and selected characteristics among children aged 4–17 years: United States, 2011–2013, NCHS Data Brief 201 (2015) 201.
[11] J.H. Park, Y.R. Bang, C.K. Kim, Sex and age differences in psychiatric disorders among children and adolescents: high-risk students study, Psych. Investig. 11 (2014) 251–257.
[12] I. Schubert, I. Köster, G. Lehmkuhl, The changing prevalence of attention-deficit/hyperactivity disorder and methylphenidate prescriptions: a study of data from a random sample of insurees of the AOK health insurance company in the German State of Hesse, 2000–2007, Dtsch Arztebl Int. 107 (2010) 615–621.
[13] S.L. Andersen, A. Arvanitogiannis, A.M. Pliakas, C. LeBlanc, W.A. Carlezon, Altered responsiveness to cocaine in rats exposed to methylphenidate during development, Nat. Neurosci. 5 (2002) 13–14.
[14] J.A. Bethancourt, C.E. Vásquez, G.B. Britton, Sex-dependent effects of long-term oral methylphenidate treatment on spontaneous and learned fear behaviors, Neurosci. Lett. 496 (2011) 30–34.
[15] L. Hrubá, B. Schutová, R. Šlamberová, Sex differences in anxiety-like behavior and locomotor activity following prenatal and postnatal methamphetamine exposure in adult rats, Physiol. Behav. 105 (2012) 364–370.
[16] S.L. Andersen, Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci. Biobehav. Rev. 27 (2003) 3–18.
[17] J.N. Giedd, F.M. Lalonde, M.J. Celano, S.L. White, G.L. Wallace, N.R. Lee, R.K. Lenroot, Anatomical brain magnetic resonance imaging of typically developing children and adolescents, J. Am. Acad. Child Adolesc. Psychiatry 48 (2009) 465–470.
[18] M. Butz, F. Wörgötter, A. van Ooyen, Activity-dependent structural plasticity, Brain Res. Ver. 60 (2009) 287–305.
[19] M.S. Izvol'skaia, E.I. Adamskaia, S.N. Voronova, A. Duittoz, I. Tillet, Catecholamines in regulation of development of GnRH neurons of rat fetuses, Ontogenez 36 (2005) 440–448.
[20] C. Fabre-Nys, Steroid control of monoamines in relation to sexual behaviour, Rev. Reprod. 3 (1998) 31–41.
[21] L.P. Spear, The adolescent brain and age-related behavioral manifestations, Neurosci. Biobehav. Ver. 24 (2000) 417–463.
[22] A.E. Herbison, Noradrenergic regulation of cyclic GnRH secretion, Rev. Reprod. 2 (1997) 1–6.
[23] X. Liu, A.E. Herbison, Dopamine regulation of gonadotropin-releasing hormone neuron excitability in male and female mice, Endocrinology 154 (2013) 340–350.
[24] A.E. Herbison, Estrogen positive feedback to gonadotropin-releasing hormone (GnRH) neurons in the rodent: the case for the rostral periventricular area of the third ventricle (RP3V), Brain Res. Ver. 57 (2008) 277–287.
[25] B. Venegas-Meneses, J.F. Padilla, C.E. Juárez, J.L. Morán, C. Morán, N.H. Rosas-Murrieta, A. Handal, R. Domínguez, Effects of ovarian dopaminergic receptors on ovulation, Endocrine 50 (2015) 783–796.
[26] H. Ferrero, C.M. García-Pascual, R. Gómez, F. Delgado-Rosas, O. Cauli, C. Simón, F. Gaytán, A. Pellicer, Dopamine receptor 2 activation inhibits ovarian vascular endothelial growth factor secretion in vitro: implications for treatment of ovarian hyperstimulation syndrome with dopamine receptor 2 agonists, Fertil. Steril. 101 (2014) 1411–1418.
[27] F. Parillo, M. Maranesi, F. Mignini, L. Marinelli, A. Di Stefano, C. Boiti, M. Zerani, Evidence for a dopamine intrinsic direct role in the regulation of the ovary reproductive function: in vitro study on rabbit corpora lutea, PLoS One 9 (2014), e104797.
[28] B. Reichow, F.R. Volkmar, M.H. Bloch, Systematic review and meta-analysis of pharmacological treatment of the symptoms of attention-deficit/hyperactivity disorder in children with pervasive developmental disorders, J. Autism Dev. Disord. 43 (2013) 2435–2441.
[29] M.H. Sibley, A.B. Kuriyan, S.W. Evans, J.G. Waxmonsky, B.H. Smith, Pharmacological and psychosocial treatments for adolescents with ADHD: an updated systematic review of the literature, Clin. Psychol. Ver. 34 (2014) 218–232.
[30] B.G. Montagnini, L.S. Silva, A.H. dos Santos, J.A. Anselmo-Franci, G.S. Fernandes, S.F. Mesquita, D.C. Gerardin, Effects of repeated administration of methylphenidate on reproductive parameters in male rats, Physiol. Behav. 133 (2014) 122–129.
[31] E.V. Marco, W. Adriani, L. Ruocco, R. Canese, A.G. Sadile, G. Laviola, Neurobehavioral adaptations to methylphenidate: the issue of early adolescent, Neurosci. Biobehav. Rev. 35 (2011) 1722–1739.
[32] S. Chatterjee-Chakrabarty, B.T. Miller, T.J. Collins, M. Nagamani, Adverse effects of methylphenidate on the reproductive axis of adolescent female rats, Fertil. Steril. 84 (2005) 1131–1138.
[33] L. Derouiche, M. Keller, M. Martini, A.H. Duittoz, D. Pillon, Developmental exposure to ethinylestradiol affects reproductive physiology, the GnRH neuroendocrine network and behaviors in female mouse, Front. Neurosci. 9 (2015) 1–14.
[34] N. Simon, B. Rolland, L. Karila, Methylphenidate in adults with attention deficit hyperactivity disorder and substance use disorders, Curr. Pharm. Des. 21 (2015) 3359–3366.
[35] M.V. Solanto, Clinical psychopharmacology of AD/HD: implications for animal models, Neurosci. Biobehav. Ver. 24 (2000) 27–30.

[36] U.S. EPA, Harmonization in Interspecies Extrapolation: Use of BW 3/4 as Default Method in Derivation of the Oral RfD (External Review Draft), U.S. Environmental Protection Agency, Washington, D.C., 2006 [EPA/630/R-06/001].
[37] D.A. Beckman, M. Schneider, M. Youreneff, F.L. Tse, Juvenile toxicity assessment of d,l-methylphenidate in rats, Birth Defects Res. B Dev. Reprod. Toxicol. 83 (2008) 48–67.
[38] R.H. Gallavan Jr., J.F. Holson, D.G. Stump, J.F. Knapp, V.L. Reynolds, Interpreting the toxicologic significance of alterations in anogenital distance: potential for confounding effects of progeny body weights, Reprod. Toxicol. 13 (1999) 383–390.
[39] J.M. Goldman, A.S. Murr, R.L. Cooper, The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies, Birth Defects Res. B Dev. Reprod. Toxicol. 80 (2007) 84–97.
[40] V.A. Chistyakov, S.V. Dem'yanenko, A.A. Alexandrova, L.V. Gutnikova, V.N. Prokof'ev, O.N. Kosheleva, Effect of plastoquinone derivative 10-(6′-plastoquinonyl) decyltriphenylphosphonium (SkQ1) on estrous cycle and 17β-estradiol level in rats, Biochem. Biokhimiîa 77 (2012) 1382–1386.
[41] T. Pedersen, H. Peters, Proposal for a classification of oocytes and follicles in the mouse ovary, J. Reprod. Fertil. 17 (1968) 555–557.
[42] C.N. de Azevedo, M.L. Vieira, B.G. Montagnini, A.C. Kiss, D.C. Gerardin, Effects of maternal exposure to the galactagogue Sulpiride on reproductive parameters in female rats, Physiol. Behav. 140 (2015) 247–253.
[43] O.A. Ramirez, H.F. Carrer, G. Nasello, Prenatal amphetamine exposure: ovulation, sexual behavior and hypothalamic monoamine content in rats, Pharmacol. Biochem. Behav. 11 (1979) 605–609.
[44] F.A. Beach, Prolonged hormone deprivation and pretest cage adaptation as factors affecting the display of lordosis by female rats, Physiol. Behav. 16 (1976) 807–808.
[45] D.F. Hardy, J.F. Debold, Effects of mounts without intromission upon the behavior of female rats during the onset of estrogen-induced heat, Physiol. Behav. 7 (1971) 643–645.
[46] E.B. Ottoni, EthoLog 2.2: a tool for the transcription and timing of behavior observation sessions, Behav. Res. Methods Instrum. Comput. 32 (2000) 446–449.
[47] B.A. Faraj, Z.H. Israili, J.M. Perel, M.L. Jenkins, S.G. Holtzman, S.A. Cucinell, P.G. Dayton, Metabolism and disposition of methylphenidate-14C: studies in man and animals, J. Pharmacol. Exp. Ther. 191 (1974) 535–547.
[48] M.R. Meyer, H.H. Maurer, Absorption, distribution, metabolism and excretion pharmacogenomics of drugs of abuse, Pharmacogenomics 12 (2011) 215–233.
[49] Z. Sun, D.J. Murry, S.P. Sanghani, W.I. Davis, N.Y. Kedishvili, Q. Zou, T.D. Hurley, W.F. Borson, Methylphenidate is stereoselectively hydrolyzed by human carboxylesterase CES1A1, J. Pharmacol. Exp. Ther. 310 (2004) 469–476.
[50] A. Balcioglu, J.Q. Ren, D. McCarthy, T.J. Spencer, J. Biederman, P.G. Bhide, Plasma and brain concentrations of oral therapeutic doses of methylphenidate and their impact on brain monoamine content in mice, Neuropharmacology 57 (2009) 687–693.
[51] M.R. Gerasimov, M. Franceschi, N.D. Volkow, A. Gifford, S.J. Gatley, D. Marsteller, P.E. Molina, S.L. Dewey, Comparison between intraperitoneal and oral methylphenidate administration: a microdialysis and locomotor activity study, J. Pharmacol. Exp. Ther. 295 (2000) 51–57.
[52] J.M. Swanson, N.D. Volkow, Pharmacokinetic and pharmacodynamic properties of stimulants: implications for the design of new treatments for ADHD, Behav. Brain Res. 130 (2002) 73–78.
[53] A. Poulton, C.T. Cowell, Slowing of growth in height and weight on stimulants: a characteristic pattern, J. Paediatr. Child Health 39 (2003) 180–185.
[54] R. Ptacek, H. Kuzelova, Methylphenidate and growth in ADHD children, Act. Nerv. Super. Rediviva 53 (2011) 45–48.
[55] A. Cansu, O. Ekinci, O. Ekinci, A. Serdaroglu, D. Erdogan, Z.K. Coskun, S.G. Gürgen, Methylphenidate has dose-dependent negative effects on rat spermatogenesis: decreased round spermatids and testicular weight and increased p53 expression and apoptosis, Hum. Exp. Toxicol. 30 (2011) 1592–1600.
[56] G.B. Britton, J.Á. Bethancourt, Characterization of anxiety-related responses in male rats following prolonged exposure to therapeutic doses of oral methylphenidate, Pharmacol. Biochem. Behav. 93 (2009) 451–459.
[57] G.C. Kennedy, J. Mitra, Body weight and food intake as initiating factors for puberty in the rat, J. Physiol. 166 (1963) 408–418.
[58] A. Holehan, B. Merry, The control of puberty in the dietary restricted female rat, Mech. Ageing Dev. 32 (1985) 179–191.
[59] C.A. Frye, E. Bo, G. Calamandrei, L. Calzà, F. Dessì-Fulgheri, M. Fernández, L. Fusani, O. Kah, M. Kajta, Y. Le Page, H.B. Patisaul, A. Venerosi, A.K. Wojtowicz, G.C. Panzica, Endocrine disrupters: a review of some sources, effects, and mechanisms of actions on behaviour and neuroendocrine systems, J. Neuroendocrinol. 24 (2012) 144–159.
[60] S.A. Berenbaum, A.M. Beltz, Sexual differentiation of human behavior: effects of prenatal and pubertal organizational hormones, Front. Neuroendocrinol. 32 (2011) 183–200.
[61] C. Wei, E.C. Crowne, Recent advances in the understanding and management of delayed puberty, Arch. Dis. Child. 101 (2015) 481–488.
[62] J. Clarkson, Effects of estradiol on kisspeptin neurons during puberty, Front. Neuroendocrinol. 34 (2013) 120–131.
[63] J.A. Maffucci, A.C. Gore, Chapter 2 hypothalamic neural systems controlling the female reproductive life cycle, Int. Rev. Cell Mol. Biol. 274 (2009) 69–127.
[64] M. Ciechanowska, M. Lapot, K. Mateusiak, F. Przekop, Neuroendocrine regulation of GnRH release and expression of GnRH and GnRH receptor genes in the hypothalamus-pituitary unit in different physiological states, Reprod. Biol. 2 (2010) 85–124.
[65] G.H. Greeley, J.S. Kizer, The effects of chronic methylphenidate treatment on growth and endocrine function in the developing rat, J. Pharmacol. Exp. Ther. 215 (1980) 545–551.
[66] R.S. Sellers, D. Morton, B. Michael, N. Roome, J.K. Johnson, B.L. Yano, R. Perry, K. Schafer, Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology studies, Toxicol. Pathol. 35 (2007) 751–755.
[67] J.E. Hart, Pituitary-related weight changes affecting the liver, uterus and adrenal glands of rats treated with hexoestrol and clomiphene in high doses, Toxicology 61 (1990) 185–194.
[68] B.G. Montagnini, S. Bortolan, B.D. dos Santos, A.P. Moreno, A. Camin Nde, D.C. Gerardin, E.G. Moreira, Evaluation of escitalopram, sertraline, and methylphenidate in the immature rat uterotrophic assay, Int. J. Toxicol. 32 (2013) 426–430.
[69] S. Teo, D. Stirling, S. Thomas, A. Hoberman, A. Kiorpes, V. Khetani, A 90-day oral gavage toxicity study of D-methylphenidate and D,L-methylphenidate in Sprague-Dawley rats, Toxicology 179 (2002) 183–196.
[70] A.S. Ferguson, S.Y. Boctor, Cocaine responsiveness or anhedonia in rats treated with methylphenidate during adolescence, Neurotoxicol. Teratol. 32 (2010) 432–442.
[71] G.A. Wood, J.E. Fata, K.L. Watson, R. Khokha, Circulating hormones and estrous stage predict cellular and stromal remodeling in murine uterus, Reproduction 133 (2007) 1035–1044.
[72] OECD/OCDE, Guideline for the Testing of Chemicals no 440: Uterotrophic Bioassay in Rodents, OECD Publishing, 2007.
[73] D.S. Stolzenberg, M. Numan, Hypothalamic interaction with the mesolimbic DA system in the control of the maternal and sexual behaviors in rats, Neurosci. Biobehav. Ver 35 (2011) 826–847.
[74] M.P. Leussis, T.L. Bond, C.M. Hawken, R.E. Brown, Attenuation of maternal behavior in virgin CD-1 mice by methylphenidate hydrochloride, Physiol. Behav. 95 (2008) 395–399.
[75] R.A. Ponchio, E. Teodorov, T.B. Kirsten, C.P. Coelho, A. Oshiro, J.C. Florio, M.M. Bernardi, Repeated methylphenidate administration during lactation reduces maternal behavior, induces maternal tolerance, and increases anxiety-like behavior in pups in adulthood, Neurotoxicol. Teratol. 50 (2015) 64–72.
[76] R. Slamberová, P. Charousová, M. Pometlová, Maternal behavior is impaired by methamphetamine administered during pre-mating, gestation and lactation, Reprod. Toxicol. 20 (2005) 103–110.
[77] E.M. Vernotica, J.S. Rosenblatt, J.I. Morrell, Microinfusion of cocaine into the medial preoptic area or nucleus accumbens transiently impairs maternal behavior in the rat, Behav. Neurosci. 113 (1999) 377–390.
[78] H.V. Peeke, K.A. Dark, A. Salamy, M. Salfi, S.N. Shah, Cocaine exposure prebreeding to weaning: maternal and offspring effects, Pharmacol. Biochem. Behav. 48 (1994) 403–410.
[79] N.D. Volkow, G.J. Wang, J.S. Fowler, M. Fischman, R. Foltin, N.N. Abumrad, S.J. Gatley, J. Logan, C. Wong, A. Gifford, Y.S. Ding, R. Hitzemann, N. Pappas, Methylphenidate and cocaine have a similar in vivo potency to block dopamine transporters in the human brain, Life Sci. 65 (1999) 7–12.
[80] J.S. Fowler, C. Kroll, R. Ferrieri, D. Alexoff, J. Logan, S.L. Dewey, W. Schiffer, D. Schlyer, P. Carter, P. King, C. Shea, Y. Xu, L. Muench, H. Benveniste, P. Vaska, N.D. Volkow, PET studies of D-methamphetamine pharmacokinetics in primates: comparison with L-methamphetamine and (−)-cocaine, J. Nucl. Med. 48 (2007) 1724–1732.
[81] D.K. Shahrokh, T.Y. Zhang, J. Diorio, A. Gratton, M.J. Meaney, Oxytocin-dopamine interactions mediate variations in maternal behavior in the rat, Endocrinology 151 (2010) 2276–2286.
[82] D.L. Robinson, D.L. Zitzman, S.K. Williams, Mesolimbic dopamine transients in motivated behaviors: focus on maternal behavior, Front Psych. 2 (2011) 23.
[83] D.E. Olazábal, E. Abercrombie, J.S. Rosenblatt, J.I. Morrell, The content of dopamine, serotonin, and their metabolites in the neural circuit that mediates maternal behavior in juvenile and adult rats, Brain Res. Bull. 63 (2004) 259–268.
[84] A.S. Thomas, R.D. Palmiter, Impaired maternal behavior in mice lacking norepinephrine and epinephrine, Cell 91 (1997) 583–592.