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Semax, synthetic ACTH(4–10) analogue, attenuates behavioural and neurochemical alterations following early-life fluvoxamine exposure in white rats

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ABSTRACT

Selective serotonin reuptake inhibitors (SSRI) are commonly used to treat depression during pregnancy. SSRIs cross the placenta and may influence the maturation of the foetal brain. Clinical and preclinical findings suggest long-term consequences of SSRI perinatal exposure for the offspring. The mechanisms of SSRI effects on developing brain remain largely unknown and there are no directional approaches for prevention of the consequences of maternal SSRI treatment during pregnancy. The heptapeptide Semax (MEHFPGP) is a synthetic analogue of ACTH(4–10) which exerts marked nootropic and neuroprotective activities. The aim of the present study was to investigate the long-term effects of neonatal exposure to the SSRI fluvoxamine (FA) in white rats. Additionally, the study examined the potential for Semax to prevent the negative consequences of neonatal FA exposure. Rat pups received FA or vehicle injections on postnatal days 1–14, a time period equivalent to 27–40 weeks of human foetal age. After FA treatment, rats were administered with Semax or vehicle on postnatal days 15–28. During the 2nd month of life, the rats underwent behavioural testing, and monoamine levels in brain structures were measured. It was shown that neonatal FA exposure leads to the impaired emotional response to stress and novelty and delayed acquisition of food-motivated maze task in adolescent and young adult rats. Furthermore, FA exposure induced alterations in the monoamine levels in brains of 1- and 2- month-old rats. Semax administration reduced the anxiety-like behaviour, improved learning abilities and normalized the levels of brain biogenic amines impaired by the FA exposure. The results demonstrate that early-life FA exposure in rat pups produces long-term disturbances in their anxiety-related behaviour, learning abilities, and brain monoamines content. Semax exerts a favourable effect on behaviour and biogenic amine system of rats exposed to the antidepressant. Thus, peptide Semax can prevent behavioural deficits caused by altered 5-HT levels during development.

1. Introduction

According to recent data, 10 to 20% of pregnant women suffer from depression with various degrees of severity (Hutchison et al., 2019). The most commonly used drugs to treat depression in pregnant and nursing women are selective serotonin reuptake inhibitors (SSRI) such as fluoxetine, citalopram, fluvoxamine, paroxetine, sertraline, etc. (Gemmel et al., 2018; Grieb and Ragan, 2019). These drugs bind to the serotonin transporter (SERT) responsible for removal of the neurotransmitter from the synaptic cleft. Inhibition of SERT causes extracellular intrasynaptic serotonin levels to increase (Millard et al., 2017). However, SSRIs affect not only the brain biogenic amine levels

Abbreviations: ACTH, adrenocorticotropic hormone; BDNF, brain-derived neurotrophic factor; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EPM, elevated plus maze; FA, fluvoxamine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; NE, norepinephrine; PA, passive avoidance; PND, postnatal day; SERT, serotonin transporter; SSRI, selective serotonin reuptake inhibitor.

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but also the synthesis of certain neuropeptides (Rovin et al., 2012). The currently available evidence suggests that the mechanism of SSRI action is complex and the antidepressant effects may be partially mediated by neuropeptides (Gołyszny and Obuchowicz, 2019). SSRIs have been shown to freely cross the placental barrier and are detected in the amniotic fluid and cord blood (Noorlander et al., 2008; Rotem-Kohavi et al., 2019). Thus, developing foetus becomes exposed to antidepressants, which can lead to imbalanced foetal serotonin (5-HT) (Rampono et al., 2009). During early embryogenesis, 5-HT is not only a neurotransmitter, but also a neurotrophic factor that regulates the growth, differentiation, migration, and myelination of neurons, as well as the synaptogenesis (Gaspar et al., 2003). Alterations in the 5-HT levels during the embryonic period can have long-term consequences for brain development and functions (Rotem-Kohavi et al., 2019). A number of clinical studies have shown a negative effect of SSRIs on pregnancy resulting in adverse outcomes: increased risk of premature birth, low birth weight, neonatal cardiovascular abnormalities, and persistent pulmonary hypertension (Hutchison et al., 2018; Olivier et al., 2011). Prenatal SSRI exposure is associated with an increased risk of mental and behavioural disorders such as autism spectrum disorder, attention-deficit/hyperactivity disorder, and mental disability (Ramsteijn et al., 2020). Nevertheless, the results of studies on the effects of antidepressant perinatal exposure on child's development are controversial (Hviid et al., 2013; Oberlander et al., 2009, 2010). The heterogeneity of available clinical data (varying in the type and dosage of SSRI prescribed, the timing of maternal treatment, age, and method of assessment of the children) makes it difficult to evaluate the consequences of SSRI effects on developing organism (Alwan and Friedman, 2009). Furthermore, distinguishing the impact of prenatal SSRI exposure from the impact of maternal depression on foetal development is challenging. As known, untreated maternal depression negatively affects the pregnancy outcome, as well as it can induce disturbances of normal child's behaviour, cognitive delays, and increased anxiety (Gemmel et al., 2018; Gentile, 2017; Hutchison et al., 2018). Studies of the own effects of maternal depression and the effects of perinatal SSRI exposure will help assess the risks and benefits of the SSRI usage during pregnancy (Olivier et al., 2013). Animal experiments become an important approach to exclude the effects of maternal depression and to control the effects of various environmental factors (Alwan and Friedman, 2009; Houwing et al., 2019). Moreover, animal experiments (mainly on rodents) allow us to explore long-term effects of SSRI exposure during pregnancy, which are poorly studied in human (Ramsteijn et al., 2020).

Because of the altricial nature of rodents, in terms of the brain development in general and 5-HT system in particular, the first postnatal weeks in the rodent correspond to the 3rd trimester of pregnancy in human (Altieri et al., 2015; Meyer et al., 2018; Millard et al., 2017; Shah et al., 2018). Therefore, SSRI administration to neonatal rats is considered as a model of prenatal exposure to antidepressants during the last trimester of human pregnancy. This period (the first 14 days of postnatal development in rats and the 3rd trimester of human pregnancy) is characterized by a rapid brain growth and development of the 5-HT system, which makes them more vulnerable to the effects of antidepressants (Millard et al., 2017; Shah et al., 2018).

As animal experiments have shown, a perinatal administration of SSRI induces long-term behavioural disorders in rodents. Early life antidepressant exposure causes impaired motor development, increased anxiety and depressive-like behaviours in adult animals (Altieri et al., 2015; de Deiró et al., 2008; Glazova et al., 2014; Gobinath et al., 2016; Ko et al., 2014), as well as leads to impaired social interaction and play behaviour in juveniles (Bond et al., 2020; Khatri et al., 2014; Simpson et al., 2011; Zimmerberg and Germeyan, 2015). A number of previously published studies did not reveal any sex-differences in the effects of perinatal SSRI exposure (Ansorge et al., 2004, 2008; Glazova et al., 2014; Rebello et al., 2014), but other studies showed some sex-specific effects of perinatal SSRIs (Gobinath et al., 2016, 2017; Pawluski and Gemmel, 2018; Goel and Bale, 2010). The findings regarding the effects of gender on the response to antidepressant remain controversial.

Behavioural disorders in adult animals caused by early SSRI exposure may be associated with alterations in the brain 5-HT system (Kinast et al., 2013; Maciag et al., 2006; Weaver et al., 2010). Nevertheless, long-term changes in the brain biogenic amines system caused by perinatal SSRI exposure have not been sufficiently studied.

Most studies on the effects of perinatal SSRI administration to animals have been performed using fluoxetine, whereas other drugs from this class are used much less frequently (Ramsteijn et al., 2020). Although all SSRIs share the ability to inhibit SERT, they differ in chemical structure and have no identical secondary pharmacologic characteristics (Altieri et al., 2015; Gołyszny and Obuchowicz, 2019). Fluvoxamine (FA, Luvox), a SSRI antidepressant similar to fluoxetine in pharmacological characteristics, exhibits a higher selectivity and effectiveness, but a shorter-lasting action (Hrdina, 1991). FA is applied to treat depression in pregnant women (Gemmel et al., 2018), but the effects of its perinatal administration are not sufficiently understood. Early developmental exposure to FA has been shown to induce impaired locomotor activity and increased anxiety-related behaviour in mice (Noorlander et al., 2008; Zheng et al., 2011). As we showed earlier, a neonatal FA exposure led to an increase in the lethality rate, retardation of somatic growth, delayed maturation of motor responses, increased anxiety, and impaired learning ability in rats (Glazova et al., 2014; Volodina et al., 2014).

The widespread use of antidepressants during pregnancy necessitates further investigation into their long-term effects on developing organism (Bond et al., 2020). The possible ways to prevent or reduce the long-term negative effects of prenatal antidepressant exposure should also be searched for. However, despite the extensive array of clinical and experimental data on negative effects of perinatal SSRI exposure, there is no information on attempts to correct these effects in the dedicated literature.

Several studies have shown a relationship between melanocortin and 5-HT systems, as well as SSRI effect on the melanocortin expression (Churruca et al., 2008; Bharne et al., 2011). The heptapeptide Semax (MEHFPGP) is a synthetic analogue of the adrenocorticotropic hormone fragment ACTH(4–10). The peptide consists of the native ACTH(4–7) fragment and the C-terminal tripeptide Pro-Gly-Pro (Ashmarin et al., 1995). A number of studies have shown that Semax improves learning and memory and exerts neuroprotective activity (Ashmarin et al., 1995; Kaplan et al., 1996; Levitskaya et al., 2004, 2008; Stavchansky et al., 2011). At present, Semax has been successfully used for the clinical treatment of stroke, cerebrovascular insufficiency and optic nerve atrophy (Gusev et al., 2005; Sheremet et al., 2004). Neonatal Semax administration has been shown to reduce anxiety and improve learning abilities in adult rats (Sebentsova et al., 2005; Sukhanova et al., 2018). Furthermore, a positive modulating effect of Semax on the 5-HT system of the rodent brain has been recorded (Eremin et al., 2005).

2. The aim of the study

The aim of the present work was to study the long-term effects of chronic FA administration to neonatal rat pups on the anxiety-related behaviour, learning abilities, and levels of biogenic amines and their metabolites in the brain, as well to examine whether the effects of neonatal FA could be affected by subsequent chronic Semax administration.

3. Materials and methods

3.1. Animals and treatment

The study was carried out on Wistar rat pups of both sexes. The Protocol of the experiment was approved by the local Committee on Biomedical Research Ethics of the Lomonosov Moscow State University (no. 97a of October 30, 2019). All methods used were in compliance

with the requirements of EU Directive 2010/63/EU for animal experiments. All efforts were made to minimise the number of animals used and their suffering.

Female and male Wistar rats weighing 250–300 g were provided by the Stolbovaya breeding centre (Moscow region, Russia). The female and male rats were mated in 3:2 proportions for three days. The pregnant rats were housed individually. The day of birth was counted as postnatal day 0 (PND 0). A total of 19 rat litters were used in the study. The pups were kept together with their mothers and littermates until weaning at PND 31, after which they were separated and housed with same-sex littermates. Animals were maintained on a 12-h dark/light cycle, in a temperature-controlled environment with food and water ad libitum (except for the food restriction during maze training). On PND 1, the pups from each litter were weighed and divided into groups. Each treatment/gender group included at least one pup from each litter. All pups were weighed daily beginning on PND 1 and ending on PND 28 to determine dosing solution volumes.

Fluvoxamine maleate (FA, Sigma, USA) and heptapeptide Semax (MEHFPGP, synthesized at the Institute of Molecular Genetics, Russian Academy of Sciences) were used in the study. FA was dissolved in distilled sterile water at concentration 5 mg/ml and was injected intraperitoneally (i/p) in volume of 2 ml/kg body weight. In accordance with the body weight, the animals received the volume of the drug necessary to obtain FA at a dose of 10 mg/kg. FA was administered from PND 1 to PND 14; a stage of neural development in rodents similar to that in the third trimester of human pregnancyFA was administered from PND 1 to PND 14; a stage of neural development in rodents similar to that in the third trimester of human pregnancy (Meyer et al., 2018). Therefore, we consider our experiments as a model of prenatal FA exposure in humans. I/p administration was selected as a route of administration, as it is often used to study effects of chronic neonatal exposure to different SSRIs including FA (Ansorge et al., 2004, 2008; Glazova et al., 2014; Karpova et al., 2009; Rebello et al., 2014; Zheng et al., 2011). The FA dosage in our experiments was comparable to that reported in previous studies on neonatal SSRI exposure (Ansorge et al., 2004, 2008; Karpova et al., 2009; Maciag et al., 2006; Rebello et al., 2014; Zheng et al., 2011), and a similar dosage was used in our earlier works (Glazova et al., 2014; Volodina et al., 2014). The studies have demonstrated the persistent effects of similar dose regimens of SSRIs on physiology and behaviour after early postnatal exposure in rodents. Semax was dissolved in distilled sterile water at concentration 0.5 mg/ml and was injected intranasally (i/n) in volume of 0.1 ml/kg on PND 15–28. In accordance with the body weight, the animals received the volume of the drug necessary to obtain Semax at a dose of 0.05 mg/kg. The Semax dosage and administration route were chosen based on the previous studies demonstrating a positive Semax effects in neonatal and adult rats (Levitskaya et al., 2008; Sebentsova et al., 2005; Sukhanova et al., 2018). In the present study, we set up two series of experiments.

In the first series, 5 litters were used. The animals of each litter were divided into three groups: intact control (IC), control (CON), and Semax (SEM). The IC group was used to assess the effects of daily experimental manipulations; the SEM group, to assess the own Semax effects. The rats of the IC group were subjected to daily handling from PND 1 to PND 28 without injections. In the CON and SEM groups, the rats received i/p distilled sterile water (vehicle) injections (2 ml/kg) daily from PND 1 to PND 14. From PND 15 to PND 28, the rats of the CON group received i/n vehicle injections (0.1 ml/kg); the rats of the SEM group received i/n Semax (0.05 mg/kg/day).

To assess the level of anxiety, the rats were subjected to the elevated plus maze (EPM) test on PND 31. On the following day (PND 32), the animals were decapitated and their brain structures were extracted to measure the levels of biogenic amines and their metabolites.

In the second series of experiments, 14 litters were used. The animals of each litter were divided into three groups: control (CON), Fluvoxamine (FA), and Fluvoxamine + Semax (FA-SEM). The animals of the CON group received daily i/p vehicle injections (2 ml/kg) from PND 1 to PND 14 and then i/n vehicle (0.1 ml/kg) from PND 15 to PND 28. The FA group rats received i/p FA injections (10 mg/kg/day) from PND 1 to PND 14 and then i/n vehicle from PND 15 to PND 28. The FA-SEM group rats received i/p FA injections (10 mg/kg/day) from PND 1 to PND 14 and then i/n Semax (0.05 mg/kg/day) from PND 15 to PND 28.

During the 2nd month of life, the rats underwent the following tests: elevated plus maze (EPM) (PND 31 and PND 60), passive avoidance test (PA) (PND 34–37), food-motivated maze task (PND 42–46), and assessment of level of food motivation (PND 49). The behavioural tests were performed with intervals of at least 3 days, so that one test did not affect the behaviour in the other test. The different time-points of the tests allowed us to evaluate the anxiety-related behaviour of adolescent and young adult rats and the learning ability of adolescent animals. The evaluation of feeding behaviour soon after the "food-motivated maze task" allowed assessing the impact of level of food motivation on the acquisition of the maze task. On PND 32 or PND 61, some of the animals were decapitated and the brain structures were extracted to measure the levels of biogenic amines and metabolites.

3.2. Elevated plus maze (EPM) test

The EPM is an accepted model for examining anxiety-like behaviour in rodents (Carobrez and Bertoglio, 2005; Hiew et al., 2020). The experimental chamber of the maze consisted of two open arms (50 × 15 cm) at right angles with two closed arms (50 × 15 × 30 cm) connected to a central platform (10 × 10 cm). The maze was raised at a height of 55 cm above the floor. A video camera connected to a monitor in the adjoining room was mounted above the maze. A rat was placed in the centre of the maze and was allowed to freely explore the maze for 3 min under the surveillance of the camera. The time spent on the open and closed arms and the numbers of entries into the open and closed arms were observed on a monitor and scored by trained observers blind to the experimental groups. The experiments were set up in two modifications: (1) under low aversive even, dim light (the open arms, 44–50 lx; the closed arms, 18–20 lx); (2) under high aversive contrast bright lighting conditions (the closed arms are darkened, 8 lx; the open arms are brightly lit, 460 lx). In each modification of the test, different animals were used.

3.3. The passive avoidance (PA) test

The step-through passive avoidance test was used to assess the retention of learned behaviour (Bairy et al., 2007; Kiryanova et al., 2017). The PA apparatus (Neurobotics, Russia) consisted of a light and a dark compartment, connected by a guillotine door. On the training day (acquisition trial), rats were placed in the light compartment and allowed to enter the dark compartment. The latency time to enter was recorded. The door was closed after the animal's entrance to the dark compartment, and 0.5 mA electric foot shock was delivered through the grid floor for 3 s. Test sessions (retention trials) were performed 72 h after training sessions. After placing the animal in the light compartment, the latency to enter the dark compartment and the total time in the light compartment were recorded for up to 180 s.

3.4. The food-motivated maze task

To assess the learning ability, we used the model of route-based learning in a maze with food reinforcement. The training procedure and apparatus was the same as that used in our previous experiments (Sebentsova et al., 2005; Sukhanova et al., 2018; Volodina et al., 2014). The maze was a square chamber divided by five transparent partitions into six corridors. Each partition had a rectangular hole that is offset relative to the holes in adjacent partitions. Prior to the experiment, the animals were deprived of food for 24 h. On the first day of experiment, the rats were placed into the maze for 30 min for adaptation. On the following four days, the rats received 5 trials within a single day, with

maximum trial duration of 3 min. The latency to exit from the starting compartment, the number of correct trials (the number of cases where the animals found food reinforcement within 3 min), and the reaction time were daily scored manually by trained observers blind to the experimental groups. The animals were fed only once a day, after the learning session.

3.5. Food motivation test

To assess the level of food motivation, rats were deprived of food for 20 h. On the experiment day, a rat was placed in a clean testing cage for 5 min for adaptation, and then a Petri dish with a food pellet was placed into the cage. The following parameters were recorded during 10 min: latency to eat, weight of feed consumed, total time of feed consumption. The weight of consumed feed was determined by weighing the dish with feed before and after the test.

3.6. Measurement of levels of biogenic amines and their metabolites

To study the levels of biogenic amines in the rat brain, the animals were quickly decapitated, and their brains were extracted on ice plate. The brain structures (the frontal cortex, the hypothalamus, the hippocampus, and the striatum) were separated and weighed for further calculations. The specimens were instantly frozen in liquid nitrogen and then stored at −70 °C. Tissue samples were homogenized in a 0.1 N-perchloric acid (1,20) with 0.5 μM 3,4-dihydroxybenzoic acid as internal standard and centrifuged at 10,000*g* × 10 min (Eppendorf 5415 R, Germany). Supernatant was analyzed by high performance liquid chromatography with electrochemical detection (HPLC/ED) (Kokhan et al., 2017). Monoamine neurotransmitters –norepinephrine (NE), serotonin (5-HT), dopamine (DA), as well as their metabolites – 5-hydroxyindolacetic acid (5-HIAA), 3,4-dioxyphenylacetic acid (DOPAC) were detected by the glassy carbon electrode set at +0.85 V with Ag/AgCl reference electrode using electrochemical detector LC-4B (Bioanalytical Systems, West Lafayette, USA). The mobile phase contained 0.1 M citrate-phosphate buffer (pH 2.9), 1.85 mM 1-octanesulfonic acid, 0.27 mM ethylenediaminetetra-acetate, and 8% acetonitrile. The analyzed compounds were separated by the analytic reverse-phase column C18, 3 μm, 100 × 4 mm (Dr. Majsch GmbH, Germany). The flow rate was 1.0 ml/min. All substances were quantified by comparing the peak areas to the standard curves. The representative chromatograms are shown in Supplement (Figs. S1–S4). The results are presented as nmol/g tissue.

3.7. Statistical analysis

Statistical analysis was performed using the Statistical Package STATISTICA, version 10.0. Data were assessed for normality by the Shapiro-Wilk's test. Normally distributed data were compared using a factorial analysis of variances (ANOVA) with Group and Sex as between-subject factors or one-way ANOVA with Experimental Conditions or Litter as factors. The data of maze task acquisition were evaluated by repeated measures ANOVA with Group and Sex as between subject and Days 1–4 as within subject factors. When a main effect of Group or Sex or their interaction was found to be significant, we conducted a Fisher LSD post hoc analysis to determine inter-group differences. The data are expressed as mean ± standard error of the mean (S.E.M.). When data were not normally distributed (PA test), analyses were performed using Kruskall–Wallis one-way analysis of variance. Non-parametric data are expressed as median and interquartile range. Differences between groups were considered statistically significant at $p < 0.05$.

4. Results

In the second series, one animal died in the CON group. In the FA and FA-SEM groups, ten animals (six male, four female) of 91 rats died during FA administration. Thus, in groups of rats received FA, the mortality rate was 11%, in control rats – 1.3% ($p = 0.033$, test difference between two proportions). In the first series, no animal died during experiments in the IC, CON and SEM groups. So, our experimental manipulations did not increase pups' mortality.

4.1. Elevated plus maze (EPM)

EPM was used to assess anxiety-related behaviour in rats at PND 31 and PND 60; some of the rats were tested under bright light, and the other under dim light. The one-way ANOVA revealed a significant effect for Experimental Conditions on all recorded parameters ($F > 5.5$; $p < 0.001$) with all groups spending significantly more time in the open arms in the dim light condition compared to the bright light condition. The result indicates an increase in the animals' anxiety and fear responses under high aversive conditions.

4.1.1. Effects of experimental manipulations and Semax exposure on rats' behaviour in the EPM (PND 31)

The following three groups of rats were used in the experiments: CON, IC, and SEM. The result of the two-way ANOVA revealed no significant main effect for Sex on rat behaviour under bright light ($F_{1, 12} < 1,8$; $p > 0.20$) and dim light ($F_{1, 23} < 0.3$; $p > 0.55$), which allowed us to present the data obtained for the entire sample of animals (Fig. 1).

There were no significant main effect for Group ($F_{1, 12} < 2.2$; $p > 0.15$ and $F_{1, 12} < 1,9$; $p > 0.18$) and no significant (Group × Sex) interaction ($F_{2, 12} < 1.2$; $p > 0.25$ and $F_{2, 23} < 1.5$; $p > 0.10$) in bright and dim lighting conditions, respectively.

Thus, the assessment of the effects of experimental manipulations and Semax exposure on the behaviour of 31-day-old rats in the EPM test did not reveal significant differences between the groups under both bright and dim lighting conditions. Therefore, the experimental manipulations (daily i/p vehicle injections from PND 1 to PND 14 and i/n vehicle injections from PND 15 to PND 28), as well as daily i/n administration of Semax from PND 15 to PND 28, did not affect the anxiety level in adolescent rats.

4.1.2. Effects of FA and Semax exposure on rats' behaviour in the EPM (PND 31)

The following three groups of rats were used in the experiments: CON, FA, and FA-SEM. Two-way ANOVA revealed no significant main effect for Sex ($F_{1, 70} < 1.2$; $p > 0.25$ and $F_{1, 43} < 0.2$; $p > 0.70$) and no significant (Group × Sex) interaction ($F < 2.8$; $p > 0.10$) under bright and dim lighting conditions in EPM which allowed us to present the data obtained for the entire sample of animals (Fig. 2). Under bright light, there was a significant effect for Group on the time spent in the open arms ($F_{2, 70} = 3.24$; $p = 0.045$) and on the number of closed arms entries ($F_{2, 70} = 3.40$; $p = 0.039$), as well as there was a trend towards significance for Group effect on the number of open arms entries ($F_{2, 70} = 2.65$; $p = 0.07$).

A post hoc analysis showed a significant decrease in these parameters in the FA group compared to control values ($p < 0.025$).There were no significant differences between the FA-SEM group and the CON and FA groups; however, there was a trend towards significance in the open arms time between FA-SEM and FA groups ($p < 0.08$). Under the dim light, ANOVA showed no significant main effect for Group on rats' behaviour in the EPM ($F_{2, 43} < 1.1$; $p > 0.35$).

Thus, the neonatal FA administration increased anxiety-related behaviour in adolescent rats under conditions that provoke the anxiety and fear response. The subsequent Semax administration normalized in part the increased animals' anxiety under these conditions. Under low-stress conditions in the EPM, the behaviour of the rats neonatally exposed to FA did not differ from the control.

4.1.3. The effect of FA and Semax exposure on rats' behaviour in the EPM test (PND 60)

On PND 60, the level of anxiety in the EPM was re-assessed for the

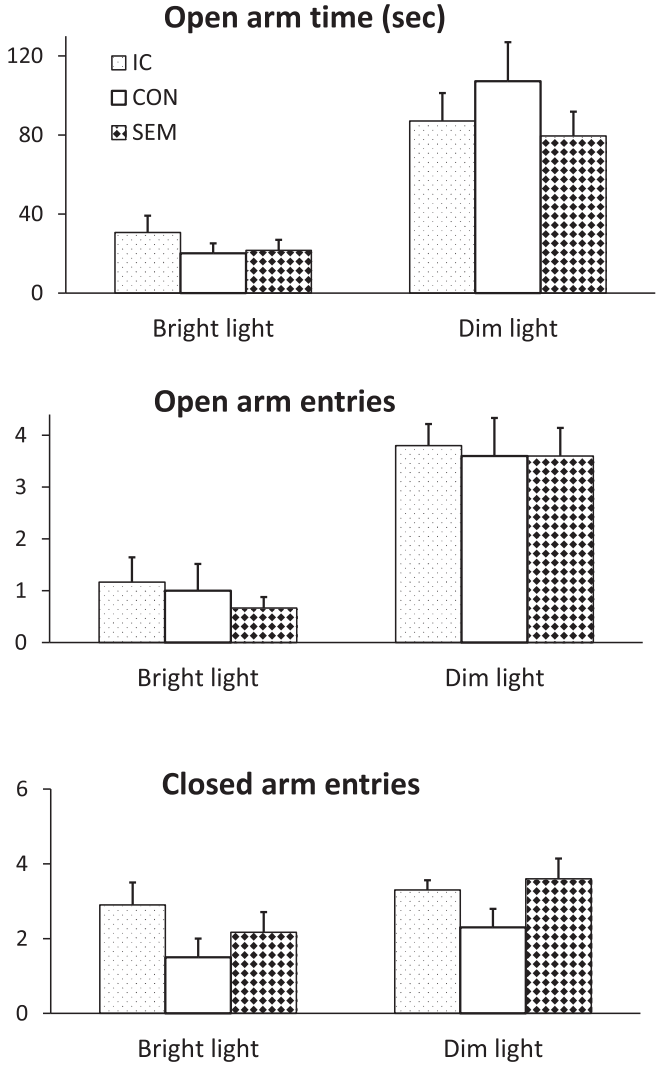

Fig. 1. Effects of the neonatal experimental manipulations and Semax administration (0.05 mg/kg/day, i/n) on the anxiety-like behaviour of adolescent rats (PND 31) in elevated plus maze under bright and dim lighting conditions. IC – intact control (un-injected), CON – control (vehicle + vehicle); SEM – (vehicle + Semax). Data were analyzed by two-way ANOVA. There were no significant Group effects. The results are expressed as mean ± S.E.M. of 6 rats per group for bright light conditions and 10 rats per group for dim light conditions.

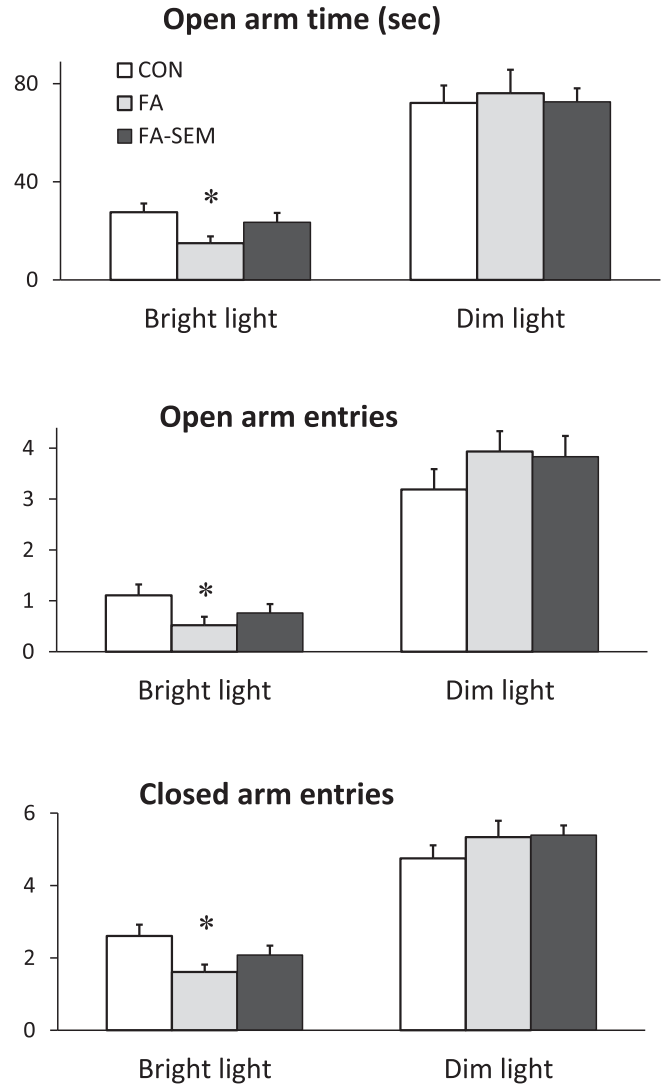

Fig. 2. Effects of neonatal FA exposure (10 mg/kg/day, i/p) and Semax treatment (0.05 mg/kg/day, i/n) on the anxiety-like behaviour of adolescent rats (PND 31) in elevated plus maze under bright and dim lighting conditions. CON – control (vehicle + vehicle); FA – (Fluvoxamine + vehicle); FA-SEM – (Fluvoxamine + Semax). Data were analyzed by two-way ANOVA followed by Fisher's LSD post hoc test.* $p < 0.05$ represents significant differences vs. corresponding control group. The results are expressed as mean ± S.E.M. of 23–25 rats per group for bright light conditions and 16–18 rats per group for dim light conditions.

rats under the same conditions as on PND 31 (conditions of bright or dim light).

The result of the two-way ANOVA revealed no significant main effect for Sex ($F_{1,\ 26} < 2.0$; $p > 0.17$ and $F_{1,\ 30} < 2.2$; $p > 0.15$) and no significant (Group × Sex) interaction ($F < 1.5$; $p > 0.24$) under bright and dim lighting conditions in EPM which allowed us to present the data obtained for the entire sample of animals (Fig. 3).

Under bright lighting conditions, there was a significant effect for Group on the time spent in the open arms ($F_{2,\ 26} = 4.73$; $p = 0.018$), as well as there was a trend towards significance for Group effect on the number of closed arms entries ($F_{2,\ 26} = 2.82$; $p = 0.08$). A post hoc analysis showed a significant decrease in the time spent in open arms and the number of the closed arms entries in the FA group compared to control values ($p < 0.001$). There were no significant differences between the FA-SEM group and the CON and FA groups; however, there was a trend towards significance in the open arms time between FA-SEM and FA groups ($p < 0.06$). Under the dim light, ANOVA showed no significant main effect for Group on rats' behaviour in the EPM ($F_{2,\ 30} < 1.0$; $p > 0.40$).

Thus, as in the age of 1 month, young adult rats neonatally exposed to FA exhibited an increased anxiety and reduced exploratory behaviour under bright lighting conditions in EPM. The Semax administration during 3–4 weeks of postnatal development partially normalized the animals' behaviour impaired by the FA exposure.

4.2. The passive avoidance (PA) test

The results of Kruskal-Wallis test revealed no significant effect for Sex on the learning parameters in PA test ($H_{1,\ N\ =50} < 1.6$, $p > 0.20$). Moreover, there were no differences between the groups in the latency to enter the dark compartment on the training day ($H_{2,\ N=50} = 3.68$, $p = 0.16$). In the retention test, there was no significant effect of Group on the latency to enter the dark compartment and the total time spent in the lit compartment ($H_{2,\ N=50} = 1.23$, $p = 0.54$ and $H_{2,\ N=50} = 0.70$, $p = 0.71$, respectively). Thus, the neonatal FA administration did not affect the

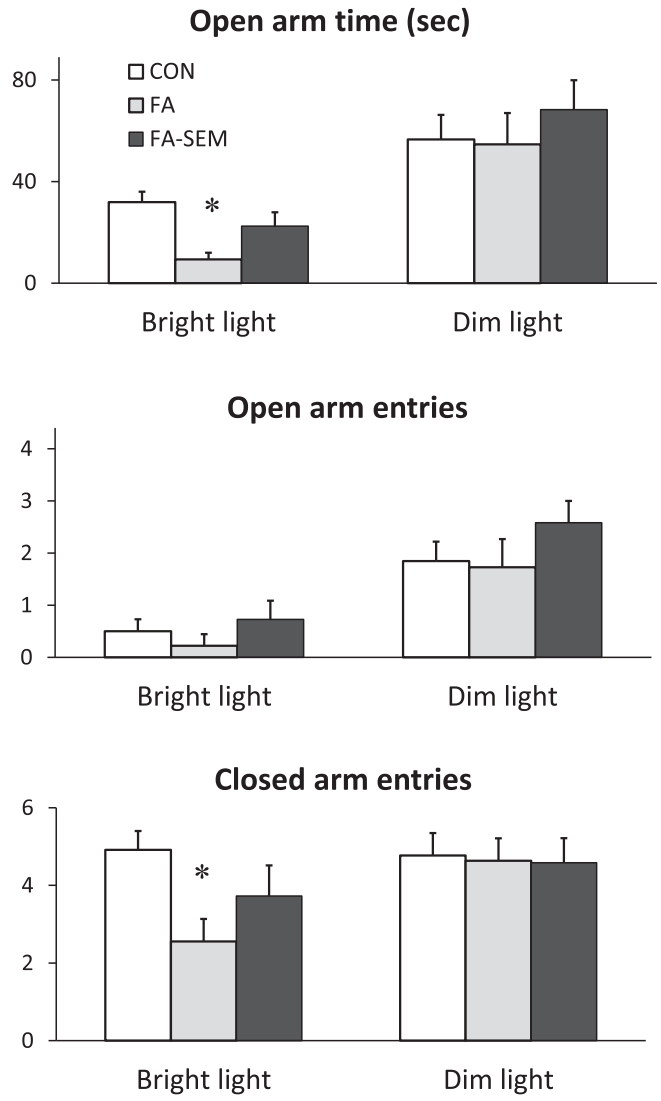

Fig. 3. Effects of neonatal FA exposure (10 mg/kg/day, i/p) and Semax treatment (0.05 mg/kg/day, i/n) on the anxiety-like behaviour of young adult rats (PND 60) in elevated plus maze under the bright and dim lighting conditions. CON – control (vehicle + vehicle); FA – (Fluvoxamine + vehicle); FA-SEM – (Fluvoxamine + Semax). Data were analyzed by two-way ANOVA followed by Fisher's LSD post hoc test. * $p < 0.05$ represents significant differences vs. corresponding control group. The results are expressed as mean ± S.E.M. of 10–12 rats per group for bright light conditions and 11–13 rats per group for dim light conditions.

acquisition of the passive avoidance task (Table 1).

4.3. The food-motivated maze task

ANOVA showed no significant main effect for Sex on the learning parameters ($F_{1,\ 63} < 1.8$, $p > 0.18$) and no significant (Group × Sex) interaction ($F_{1,\ 63} < 0.4$, $p > 0.70$), which allowed us to present the data obtained for the entire sample of animals (Fig. 4). The result of the repeated ANOVA revealed a significant main effect for Day on all the parameters recorded ($F_{3,\ 189} > 20$; $p < 0.0001$) which reflected successful learning in animals of all groups. There was a significant main effect for Group on the following parameters: latency to exit from the starting compartment ($F_{2,\ 63} = 4.0$, $p = 0.023$), reaction time ($F_{2,\ 63} = 6.0$, $p = 0.004$), and number of correct trials ($F_{2,\ 63} = 5.36$, $p = 0.007$). The post hoc analysis showed that during the first two days of training, the number of correct trials was significantly lower, whereas the latency

Table 1
Effects of the neonatal FA exposure (10 mg/kg/day, i/p) and Semax treatment (0.05 vg/kg/day, i/n) on the passive avoidance behaviour (PND 34–37).

Group (N)	Latency to enter dark compartment, acquisition trial, (sec)	Latency to enter dark compartment, retention trial, (sec)	Time spent in the lit compartment, retention trial, (sec)
CON (19)	13.0 (6.0; 55.0)	150.0 (7.0; 180.0)	173.0 (21.0; 180.0)
FA (15)	8.0 (5.0; 48.0)	45.0 (4.0; 180.0)	108.0 (32.0; 180.0)
FA-SEM (16)	15.0 (4.0; 58.0)	30.0 (8.0; 180.0)	123.0 (29.0; 180.0)

CON – control (vehicle + vehicle); FA – (Fluvoxamine + vehicle); FA-SEM – (Fluvoxamine + Semax). The number of animals per group is shown in parentheses. Data were analyzed by Kruskall–Wallis analysis. There were no significant differences between groups in this test. The results are presented as median and interquartile range.

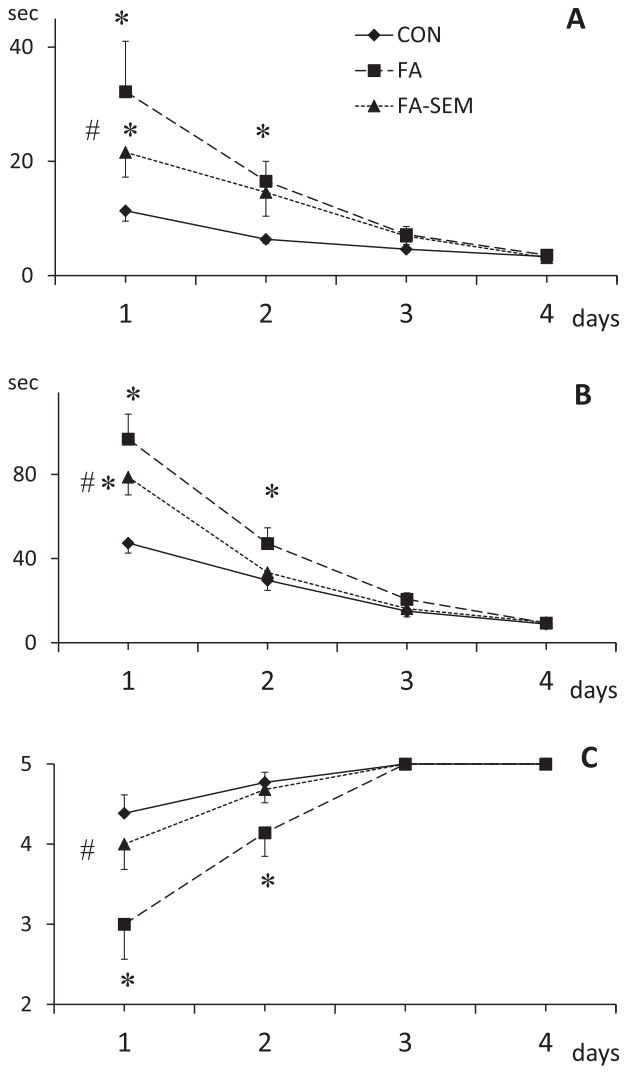

Fig. 4. Effects of neonatal FA exposure (10 mg/kg/day, i/p) and Semax treatment (0.05 mg/kg/day, i/n) on the acquisition of food-motivated maze task. X-axis – days of training; Y-axis: A – latency to exit from the starting compartment (*sec*); B – reaction time (sec); C – the number of correct trials. CON – control (vehicle + vehicle); FA – (Fluvoxamine + vehicle); FA-SEM – (Fluvoxamine + Semax). Data were analyzed by repeated measures ANOVA followed by Fisher's LSD post hoc test. * $p < 0.05$ represents significant differences vs. control group; # $p < 0.05$ represent significant differences vs. FA group. The results are expressed as mean ± S.E.M. of 10–12 rats per group.

and reaction time were significantly higher in the FA group vs. control values ($p < 0.02$). In the FA-SEM group, there was a significant increase in the latency and reaction time on the 1st training day ($p < 0.02$) compared to the control.

At the same time, a significant increase in the number of correct trials and a significant decrease in the latency and reaction time, were recorded on the 1st training day in the FA-SEM group compared to the FA group ($p < 0.02$) (Fig. 4).

The results indicate impairment in the food-motivated learning in the animals neonatally exposed to FA. The Semax administration significantly attenuated the negative FA effects.

4.4. Food motivation test

The result of two-way ANOVA revealed significant main effect for Sex on the weight of feed consumed ($F_{1, 54} = 4.98$, $p = 0.03$): in male group the weight of feed consumed was significantly greater than that in females (4.2 ± 0.17 g vs. 3.7 ± 0.17 g, respectively). ANOVA showed no significant effect for Sex on the latency to eat and total time of feed consumption ($F_{2, 54} < 0.65$, $p > 0.40$). At the same time, two-way ANOVA revealed no significant main effect for Group ($F_{2, 54} < 1.46$, $p > 0.25$) and no significant Group × Sex interaction ($F_{2, 54} < 1.06$, $p > 0.35$) for all the indications of food motivation recorded. Therefore, the neonatal FA exposure and the subsequent Semax administration do not affect the food motivation in rats (data not shown).

4.5. The levels of biogenic amines and their metabolites in the rat brain

The biogenic amine levels in brain structures were measured in 32-day-old rats from the IC, CON, and SEM groups and in 32- and 61-days-olds rats from the CON, FA, and FA-SEM groups. Two-way ANOVA showed no significant main effect for Sex and no significant Group × Sex interaction in all series of the experiments ($F < 2.5$; $p > 0.10$), which allowed us to present the results obtained for the entire sample of animals (Tables 2 and 3). The result of ANOVA revealed a significant main effect for Litter on the biogenic amine levels in all experimental series ($F > 5$; $p < 0.01$), which indicated a variability of the parameters between different litters. To reduce the effect of inter-litter variability all values of control and treatment samples were normalized by dividing them by the mean value of the respective Litter control. The further analysis was carried out with normalized data.

4.5.1. Effects of experimental manipulations and Semax administration on the brain biogenic amine levels (32 PND)

The result of ANOVA revealed no significant main effect for Group on the levels of biogenic amines and their metabolites in all brain structures studied ($F_{2, 41} < 3.00$; $p > 0.10$). Consequently, the manipulations used and the daily Semax administration during 3–4 weeks of life did not affect the levels of the brain biogenic amines in the 32-day-old rats (Fig. 5).

4.5.2. Effects of FA and Semax exposure on the brain biogenic amine levels (32 PND)

The result of ANOVA revealed no significant main effect for Group on the biogenic amines and their metabolites levels in the frontal cortex ($F_{2, 49} < 2.4$; $p > 0.10$) (Fig. 6).

In the hippocampus, the significant main effect for Group on the levels of 5-HT and its metabolite 5-HIAA was recorded ($F_{2, 50} = 5.46$; $p = 0.007$ and $F_{2, 50} = 4.72$; $p = 0.013$, respectively). A post hoc analysis showed a significant increase in the hippocampal levels of 5-HT and 5-HIAA in the FA group of rats vs. control ($p < 0.02$). The parameters in the FA-SEM group did not differ from the control values ($p > 0.25$); the hippocampal 5-HT and 5-HIAA levels in the FA-SEM group were significantly lower than the respective parameters in the FA group ($p < 0.04$). In the hypothalamus, the significant main effect for Group on the levels 5-HIAA was recorded ($F_{2, 49} = 4.65$; $p = 0.014$). A post hoc analysis revealed significant increase in hypothalamic 5-HIAA levels in the FA and FA-SEM groups vs. control ($p = 0.009$ and $p = 0.016$, respectively).

There were no differences between the FA and FA-SEM groups ($p > 0.20$). In the striatum, ANOVA revealed a significant main effect for Group on the 5-HT and 5-HIAA levels ($F_{2, 49} = 3.87$; $p = 0.028$ and $F_{2, 49} = 8.47$; $p = 0.001$, respectively). A post hoc analysis showed a significant increase in the striatal 5-HT and 5-HIAA levels in FA group vs. control ($p < 0.01$). There were no differences between the FA-SEM and

Table 2
Effects of the neonatal experimental manipulations and Semax administration (Series 1) and the neonatal FA exposure and Semax treatment (Series 2) on the levels of biogenic amines and their metabolites (nmol/g tissue) in various brain structures of adolescent rats (PND 32).

	Series 1			Series 2		
	IC (16)	CON (16)	SEM (15)	CON (18)	FA (16)	FA-SEM (19)
Frontal cortex						
NA	1.08 ± 0.17	1.00 ± 0.13	1.02 ± 0.16	1.09 ± 0.12	1.21 ± 0.15	1.19 ± 0.13
DA	0.33 ± 0.04	0.29 ± 0.05	0.30 ± 0.06	0.44 ± 0.08	0.33 ± 0.04	0.47 ± 0.08
DOPAC	0.12 ± 0.02	0.19 ± 0.05	0.12 ± 0.02	0.22 ± 0.12	0.12 ± 0.03	0.11 ± 0.02
5-HT	4.01 ± 0.53	3.60 ± 0.50	3.89 ± 0.58	4.13 ± 0.51	4.32 ± 0.54	4.63 ± 0.48
5-HIAA	0.89 ± 0.09	0.84 ± 0.09	0.83 ± 0.08	0.97 ± 0.12	0.99 ± 0.16	1.13 ± 0.12
Hippocampus						
NA	1.60 ± 0.10	1.65 ± 0.20	1.54 ± 0.14	1.67 ± 0.12	1.77 ± 0.08	1.45 ± 0.10
DA	0.12 ± 0.03	0.13 ± 0.03	0.09 ± 0.02	0.09 ± 0.03	0.14 ± 0.04	0.12 ± 0.04
DOPAC	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
5-HT	2.23 ± 0.23	2.68 ± 0.52	2.86 ± 0.62	2.54 ± 0.24	2.87 ± 0.29	2.22 ± 0.20
5-HIAA	1.23 ± 0.16	1.26 ± 0.23	1.33 ± 0.24	1.49 ± 0.17	1.84 ± 0.22	1.58 ± 0.19
Hypothalamus						
NA	11.3 ± 1.31	10.7 ± 1.05	10.8 ± 1.36	11.1 ± 0.66	11.36 ± 0.75	11.9 ± 0.93
DA	2.72 ± 0.40	3.12 ± 0.49	3.07 ± 0.52	2.75 ± 0.24	3.42 ± 0.55	2.93 ± 0.28
DOPAC	0.37 ± 0.09	0.41 ± 0.09	0.34 ± 0.07	0.45 ± 0.07	0.54 ± 0.14	0.38 ± 0.04
5-HT	12.1 ± 1.58	12.4 ± 1.56	12.2 ± 1.99	12.2 ± 1.13	12.7 ± 1.22	13.3 ± 1.23
5-HIAA	4.56 ± 0.63	4.12 ± 0.54	3.86 ± 0.64	4.56 ± 0.45	5.18 ± 0.50	5.37 ± 0.49
Striatum						
NA	0.44 ± 0.06	0.47 ± 0.08	0.62 ± 0.18	0.49 ± 0.06	0.61 ± 0.07	0.55 ± 0.05
DA	30.9 ± 1.92	32.9 ± 1.99	34.2 ± 2.24	35.7 ± 3.54	29.6 ± 1.15	28.2 ± 1.75
DOPAC	3.13 ± 0.17	3.30 ± 0.15	3.35 ± 0.13	3.71 ± 0.36	3.26 ± 0.18	3.17 ± 0.22
5-HT	2.62 ± 0.12	2.58 ± 0.07	2.46 ± 0.06	2.32 ± 0.06	2.68 ± 0.11*	2.40 ± 0.08#
5-HIAA	2.03 ± 0.13	2.15 ± 0.07	2.02 ± 0.08	1.95 ± 0.05	2.30 ± 0.07*	2.06 ± 0.06#

IC – intact control (un-injected); CON – control (vehicle + vehicle); SEM – (vehicle + Semax, 0.05 mg/kg/day, i/n); FA – (Fluvoxamine, 10 mg/kg/day, i/p + vehicle); FA-SEM – (Fluvoxamine, 10 mg/kg/day, i/p + Semax, 0.05 mg/kg/day, i/n). The number of animals per group is shown in parentheses. The results are presented as the mean ± S.E.M. Data were analyzed by two-way ANOVA followed by Fisher's LSD post hoc test. In series 1, ANOVA revealed no significant Group effects. In series 2, ANOVA showed significant main effects for Group on the 5-HT and 5-HIAA levels in the striatum ($F_{2,49} > 4.0$, $p < 0.03$).

* $p < 0.05$ represents significant differences vs. control group.

\# $p < 0.05$ – vs. FA group.

Table 3
Effects of the neonatal FA exposure (10 mg/kg/day, i/p) and Semax treatment (0.05 mg/kg/day, i/n) on the levels of biogenic amines and their metabolites (nmol/g tissue) in various brain structures of young adult rats (PND 61).

	CON (19)	FA (16)	FA-SEM (18)
Frontal cortex			
NA	0.56 ± 0.06	0.51 ± 0.06	0.54 ± 0.05
DA	0.31 ± 0.01	0.31 ± 0.03	0.33 ± 0.03
DOPAC	0.12 ± 0.01	0.14 ± 0.02	0.11 ± 0.02
5-HT	2.73 ± 0.09	2.50 ± 0.13	2.74 ± 0.07
5-HIAA	0.69 ± 0.03	0.67 ± 0.04	0.69 ± 0.03
Hippocampus			
NA	0.95 ± 0.09	1.14 ± 0.13	1.01 ± 0.10
DA	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
DOPAC	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
5-HT	2.43 ± 0.15	2.64 ± 0.14	2.51 ± 0.11
5-HIAA	1.18 ± 0.05	1.31 ± 0.05	1.26 ± 0.04
Hypothalamus			
NA	6.50 ± 0.34	7.34 ± 0.38	6.73 ± 0.42
DA	1.77 ± 0.14	1.63 ± 0.10	1.56 ± 0.12
DOPAC	0.34 ± 0.04	0.34 ± 0.06	0.33 ± 0.05
5-HT	5.38 ± 0.28	5.29 ± 0.30	5.46 ± 0.26
5-HIAA	1.87 ± 0.08	1.86 ± 0.08	2.00 ± 0.09
Striatum			
NA	0.13 ± 0.03	0.12 ± 0.03	0.11 ± 0.03
DA	46.3 ± 2.74	44.5 ± 3.05	46.2 ± 2.67
DOPAC	4.12 ± 0.33	3.93 ± 0.28	4.23 ± 0.28
5-HT	3.12 ± 0.09	3.02 ± 0.10	3.00 ± 0.12
5-HIAA	1.81 ± 0.10	1.62 ± 0.07	1.62 ± 0.07

CON – control (vehicle + vehicle); FA – (Fluvoxamine + vehicle); FA-SEM – (Fluvoxamine + Semax).
The number of animals per group is shown in parentheses. The results are presented as the mean ± S.E.M.
Data were analyzed by two-way ANOVA. The tendency for Group effects on the 5-HT levels in the frontal cortex and 5-HIAA levels in the hippocampus was revealed ($F > 2.4$, $p < 0.10$).

control groups in the parameters ($p > 0.18$); the striatal 5-HT and 5-HIAA levels in the FA-SEM group were significantly lower than in the FA group ($p < 0.05$). Thus, neonatal FA administration causes the functional activity of the 5-HT system in the hippocampus, hypothalamus, and striatum to increase in the adolescent rats. The subsequent Semax administration largely compensates for disturbances of the brain biogenic amine system caused by the neonatal FA exposure.

4.5.3. Effects of FA and Semax exposure on the brain biogenic amine levels (61 PND)

In the frontal cortex, the result of ANOVA revealed a significant main effect for Group on the 5-HT levels ($F_{2, 50} = 3.94$; $p = 0.026$) and no significant Group effects on the other parameters ($F_{2, 50} < 2.1$; $p > 0.13$) (Fig. 7). A post hoc analysis showed a significant decrease in the 5-HT levels in the FA group vs. control ($p < 0.01$).

In the FA-SEM group, the 5-HT levels in the frontal cortex did not differ from the control ($p > 0.93$), but was significantly higher than that in the FA group ($p < 0.03$). For the hippocampus, ANOVA revealed no significant main effect for Group on the parameters recorded, but there was a strong tendency for Group effect on the levels of NE ($F_{2, 49} = 3.13$; $p = 0.053$) and 5-HIAA ($F_{2, 49} = 2.87$; $p = 0.07$). A post hoc analysis showed a significant increase in the hippocampal NE and 5-HIAA levels in the FA group vs. control ($p < 0.02$). In the FA-SEM group, the parameters did not differ from the control, but there was a trend towards significance in the hippocampal 5-HIAA levels between FA-SEM and CON groups ($p < 0.08$). At the same time, the hippocampal NE levels in the FA-SEM group was significantly lower than in the FA group ($p < 0.04$). For the hypothalamus, ANOVA showed a significant main effect for Group on the NE levels only ($F_{2, 50} = 4.18$; $p = 0.049$). A post hoc comparison revealed a significant increase in the NE levels in the FA group vs. control ($p < 0.02$). In the FA-SEM group of rats, the hypothalamic NE levels did not differ from the control and FA groups ($p > 0.12$). For the striatum, the result of ANOVA showed no significant main effect for Group on the levels of biogenic amines and their metabolites ($F_{2, 50} < 0.5$; $p > 0.60$).

Thus, young adult rats neonatally exposed to FA exhibited alterations in the brain biogenic amine system: an increase in the NE levels in the hippocampus and hypothalamus, as well as a decrease in the 5-HT levels in the frontal cortex and an increase in the 5-HIAA levels in the hippocampus. The subsequent chronic Semax administration normalized in part the recorded changes.

5. Discussion

In the current study we investigated the delayed effects of chronic administration of SSRI fluvoxamine to rats from postnatal days 1 to 14. The drug was administered during the period in rat brain development which most closely resembles third trimester brain development in humans (Altieri et al., 2015; Millard et al., 2017; Shah et al., 2018). The FA exposure was shown to disturb emotional response to stress and novelty in adolescent and young adult animals, as well as impaired acquisition of the food-motivated maze task, but not passive avoidance task. Moreover, neonatal FA exposure led to the alterations in the biogenic amine levels in the brain structures of 32- and 61-day-old rats. The data obtained indicate the long-term effects of neonatal administration of the antidepressant on both the behaviour and the brain biogenic amine system. The subsequent Semax administration attenuated the negative FA effects.

The delayed effects of the experimental manipulations used and the own effects of Semax were investigated in a separate series of experiments. There were no significant differences in anxiety-like behaviour in EPM and brain biogenic amines levels between un-injected, vehicle-injected and Semax-treated groups of 1-month-old rats. Previously, we also demonstrated that i/p vehicle injections within the first 14 days of life do not affect the physical and motor development of juvenile rats and cannot cause significant changes in the brain biogenic amine levels on PND 16 (Glazova et al., 2014). Consequently, neither the experimental manipulations used nor the chronic i/n Semax administration to healthy rat pups affect development, anxiety-related behaviour, and the brain biogenic amine system in adolescent rats. Hence, we did not include IC and SEM groups in the young adult portion of the study.

Clinical studies have shown sex-related differences in the effectiveness of antidepressants, with depressed women better responding to SSRI therapy than men (Keers and Aitchison, 2010; Zammataro et al., 2017). Some animal experiments also showed the sex-specific effects of SSRI in adult rodents (Goel and Bale, 2010; Zammataro et al., 2017). The sex-differences in the effects of perinatal exposure to SSRIs remain debatable, as controversial results have been obtained. The maternal postpartum SSRI exposure was reported to have sex-dependent effects on anxiety-like behaviour, HPA axis negative feedback regulation, and hippocampal neurogenesis in offspring (Gobinath et al., 2016, 2017; Pawluski and Gemmel, 2018; Rayen et al., 2015). But direct SSRI administration to rat pups during the neonatal period did not cause sex-specific effects on several physiological and behavioural characteristics (Ansorge et al., 2004, 2008; Glazova et al., 2014; Rebello et al., 2014). In the present study, we also did not find any sex-differences in the effects of neonatal FA exposure on the behaviour and brain biogenic amine content in adolescent and young adult rats. The mixed results are likely due to the methodological differences including timing and method of administration (directly to pups or through maternal exposure) (Gobinath et al., 2016).

The effects of early-life SSRI exposure on anxiety-like and exploratory behaviour in rodents were studied by many authors (Ramsteijn et al., 2020). However, there are some discrepancies in the result of studies of the behavioural effects of SSRI neonatal exposure. To assess anxiety-related and exploratory behaviour in rodents, the standard methods are most often used: the open-field and elevated plus maze

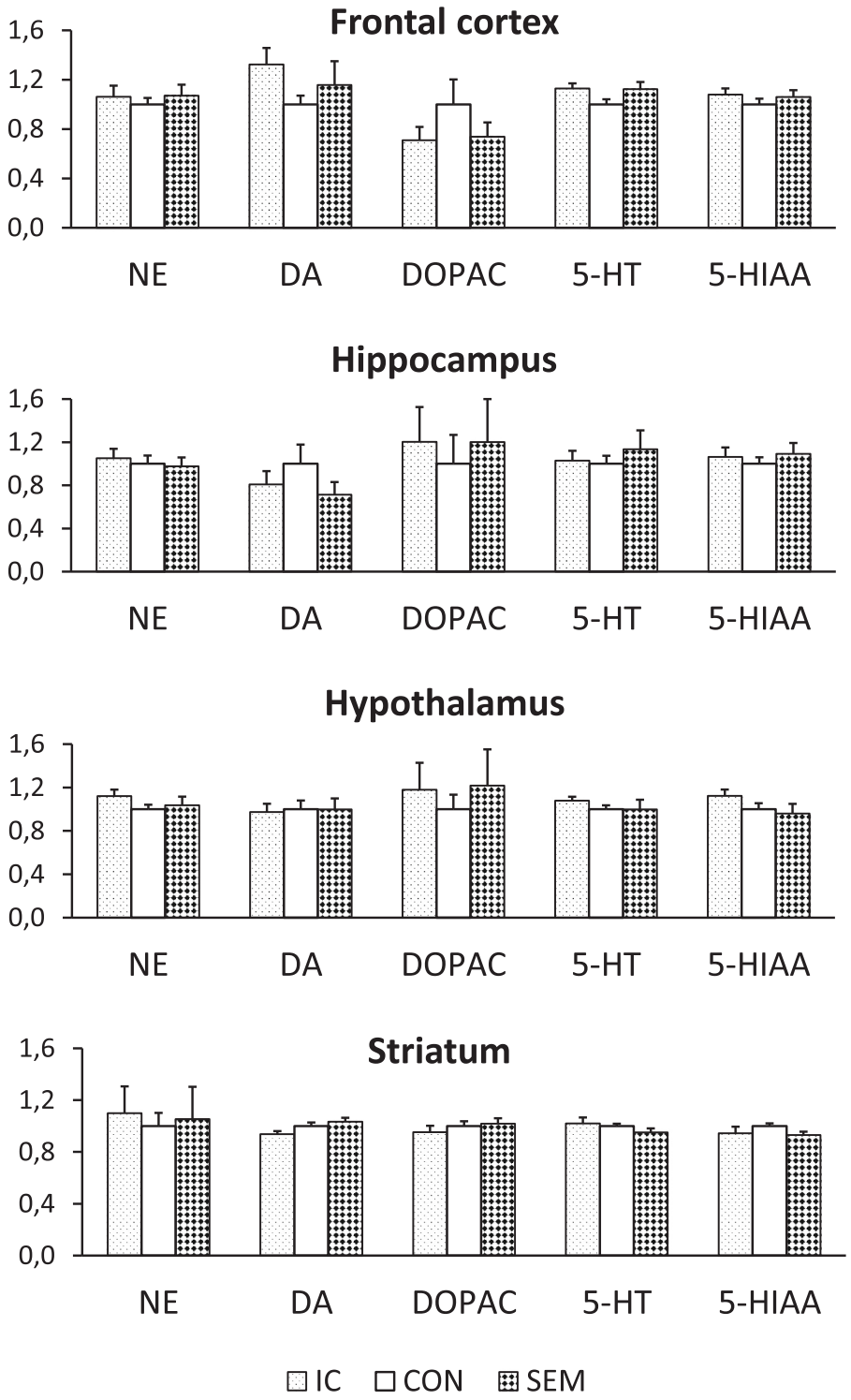

Fig. 5. Effects of the neonatal experimental manipulations and Semax administration (0.05 mg/kg/day, i/n) on the levels of biogenic amines and their metabolites in brains of adolescent rats (PND 32). IC – intact control (un-injected), CON – control (vehicle + vehicle); SEM – (vehicle + Semax). Data were analyzed by two-way ANOVA. The results are expressed as mean ± S.E.M. of 15–16 rats per group.

(EPM) tests. In many studies, neonatal exposure to SSRI decreases exploration and increases anxiety-related behaviour in rodents in the open-field test (Altieri et al., 2015; Ansorge et al., 2008; Ansorge et al., 2004; Karpova et al., 2009). However, the EPM test provides contradictory data. A number of studies report that rodents neonatally exposed to SSRI had an increase in anxiety-like behaviour in this test (Ansorge et al., 2008; Ansorge et al., 2004; Ko et al., 2014). Though, no changes in anxious behaviour were observed in other studies (Altieri et al., 2015; Harris et al., 2012; Popa et al., 2008; Ribas et al., 2008). This contradiction may result from the use of different rodent species, the differences in SSRI types, timing, and doses of exposure, and the age of animals tested. Additionally, the EPM test conditions vary between different studies. The EPM test conditions are known to determine the baseline anxiety level and, therefore, may influence the test sensitivity

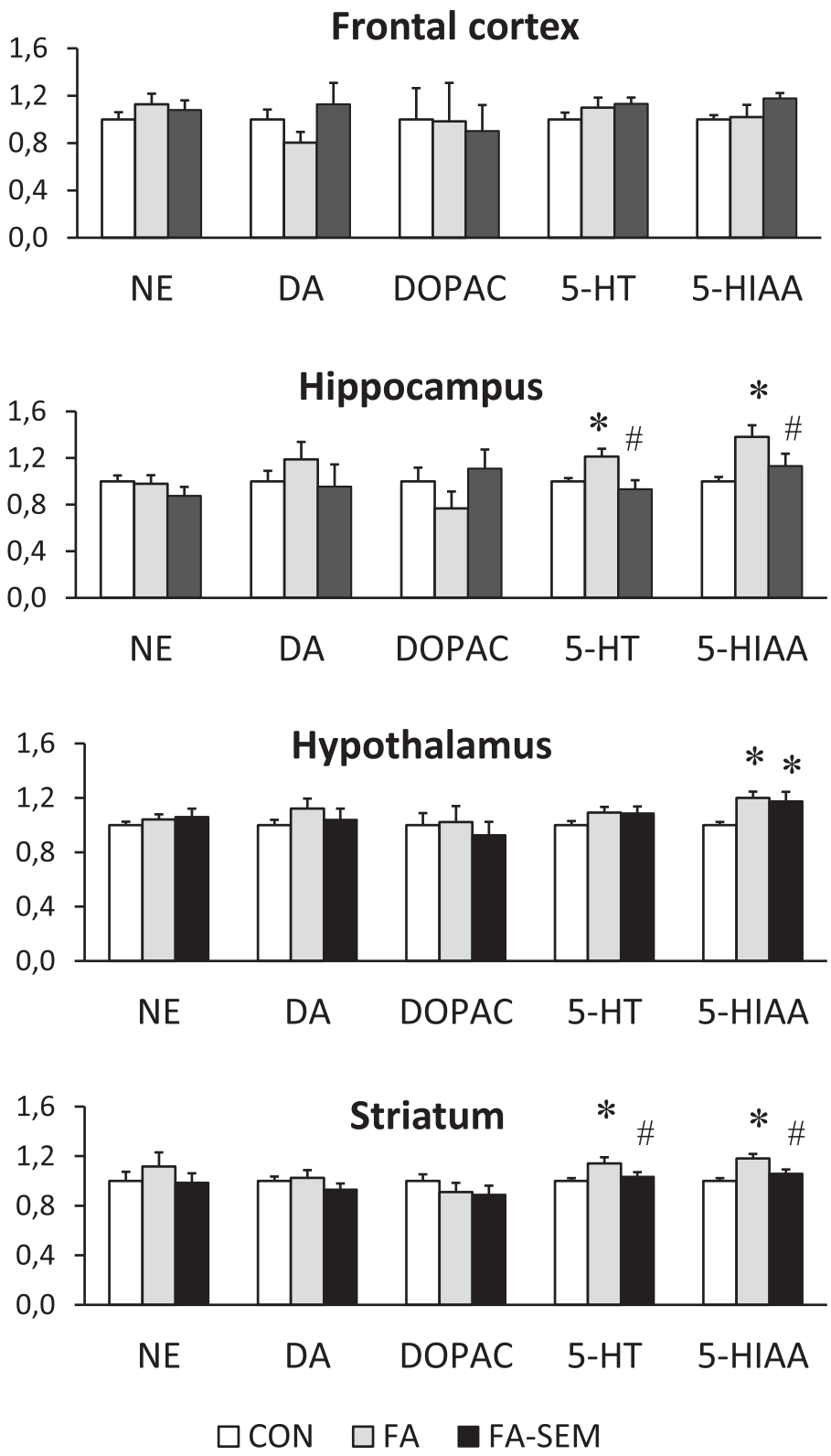

Fig. 6. Effects of neonatal FA exposure (10 mg/kg/day) and Semax treatment (0.05 mg/kg/day) on the levels of biogenic amines and their metabolites in brains of adolescent rats (PND 32). CON – control (vehicle + vehicle); FA – (Fluvoxamine + vehicle); FA-SEM – (Fluvoxamine + Semax). Data were analyzed by two-way ANOVA followed by Fisher's LSD post hoc test. * $p < 0.05$ represents significant differences vs. control group; # $p < 0.05$ represent significant differences vs. FA group. The results are expressed as mean ± S.E.M. of 16–19 rats per group.

for detecting the anxiolytic/anxiogenic effects (Altieri et al., 2015; Hiew et al., 2020; Pereira et al., 2005). In the present study, behaviour of adolescent and young adult rats was evaluated in two modifications of the EPM test. It was shown that under low aversive dim light conditions, the behaviour of experimental rats did not differ from the control. However, under high aversive bright light conditions, i.e. under conditions that provoke a fear response, the FA-exposed rats exhibited an increase in anxiety-like behaviour and a decrease in exploratory behaviour. The FA-exposed rats showed higher avoidance response to light compared to controls at both one and two month of age. Thus, the neonatal FA exposure subsequently induces an anxiogenic-like response in adolescent and young adult rats under stressful conditions, which is

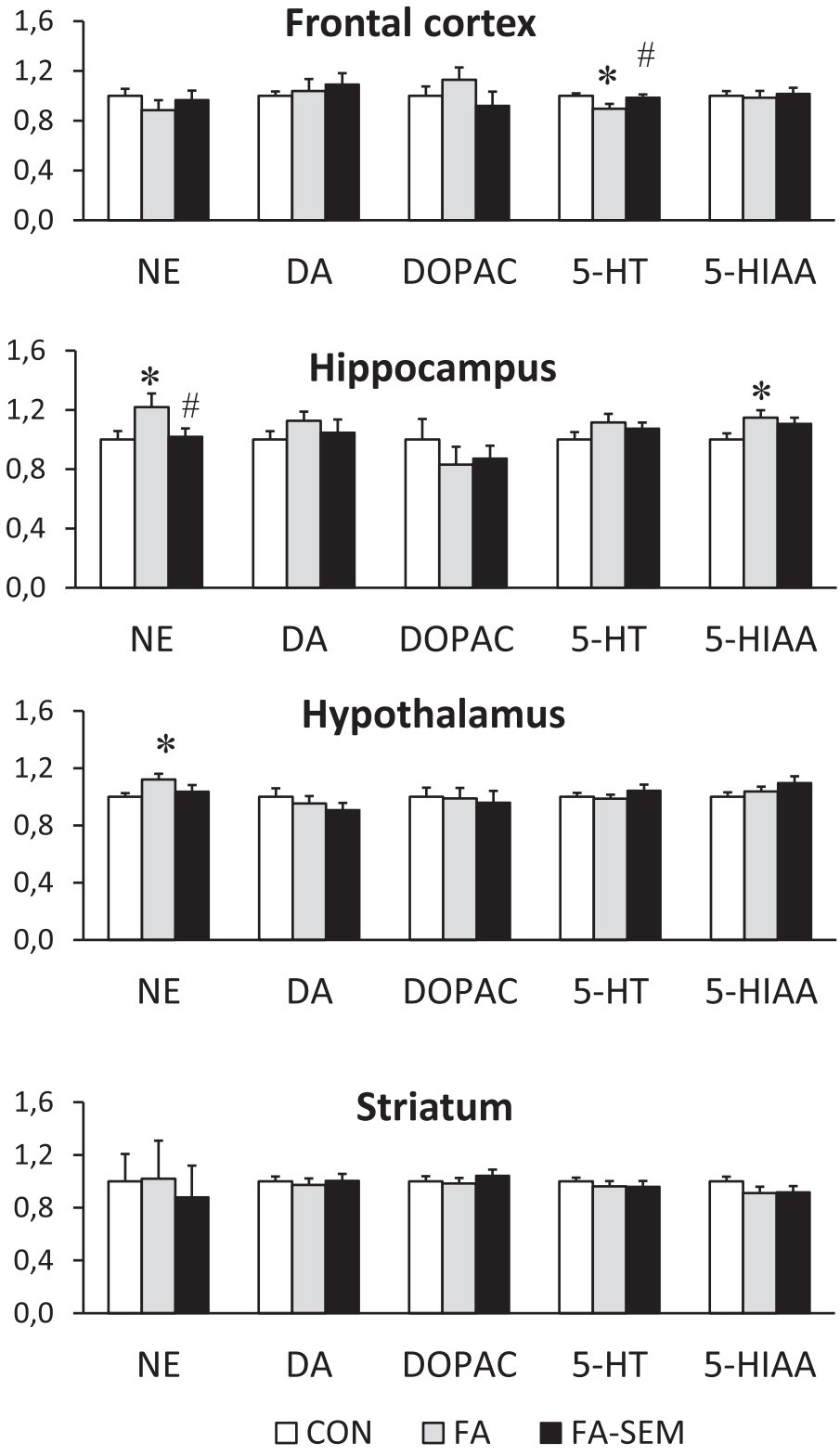

Fig. 7. Effects of neonatal FA exposure (10 mg/kg/day) and Semax treatment (0.05 mg/kg/day) on the levels of biogenic amines and their metabolites in brains of young adult rats (PND 61). CON – control (vehicle + vehicle); FA – (Fluvoxamine + vehicle); FA-SEM – (Fluvoxamine + Semax). Data were analyzed by two-way ANOVA followed by Fisher's LSD post hoc test. * $p < 0.05$ represents significant differences vs. control groups; # $p < 0.05$ represent significant differences vs. FA group (Fisher's LSD post hoc test). The results are expressed as mean ± S.E.M. of 16–18 rats per group.

associated with the impaired emotional response to stress and novelty. One of the plausible explanations for the contradictory effects of perinatal SSRI exposure on rodent behaviour in the EPM may be the differences in testing conditions.

The studies on the effect of early-life SSRI exposure on learning and memory have also shown quite contradictory results. The effects of developmental antidepressant exposure were different depending on the period of SSRI administration (Ramsteijn et al., 2020). The prenatal fluoxetine exposure had a favourable effect on learning and memory in water maze and passive avoidance test in adult rats (Bairy et al., 2007). Adult rats neonatally exposed to citalopram subsequently exhibited impairments of both spatial and egocentric learning (Schaefer et al., 2013). In studies on the effects of perinatal SSRI administration on cognitive functions, ambiguous results were obtained. Adult rats

perinatally exposed to citalopram or fluoxetine had impaired spatial learning (Sprowles et al., 2017). However, perinatal sertraline administration did not have any effect on spatial learning in mice (Meyer et al., 2018). Kiryanova and co-authors reported improvements in spatial learning, but not avoidance behaviour in adult rats perinatally exposed to fluoxetine (Kiryanova et al., 2017; Kiryanova and Dyck, 2014). In our work, we used the model of egocentric route-based learning in a maze with food reinforcement. We showed that chronic neonatal FA exposure delays the acquisition of food-motivated maze task in adult rats. The observed alterations are not associated with the impaired motor activity, since these rats did not subsequently show alterations in the overall activity in the EPM test under dim light (i.e. conditions similar to those used in food-motivated maze). Furthermore, the slowdown of learning is not related to the food motivation in rats, since no changes in animal feeding behaviour were recorded. The present experiments and literature data (Ramsteijn et al., 2020; Schaefer et al., 2013) suggest that the neonatal SSRI administration results in impaired spatial and non-spatial learning, but does not affect the avoidance behaviour in adult rodents.

Numerous studies have shown that an increase in the extracellular serotonin levels during early development causes alterations in the 5-HT system of adult animal brains (Kinast et al., 2013; Maciag et al., 2006; Weaver et al., 2010). We have reported previously the increased 5-HT turnover in brains of rats neonatally exposed to FA at 48 h after drug discontinuation (Glazova et al., 2014). In the present study, we have recorded increased 5-HT levels in the hippocampus and striatum, and also increased 5-HIAA levels in the hippocampus, striatum, and hypothalamus of the FA-exposed rats at age 32 days. This means that the increased activity of the 5-HT system in animals persists within 18 days following discontinuation of the FA treatment. No significant changes in the DA and NE levels were observed in 32 days aged rats. Studies conducted on adult rodents have shown an increase in the levels of the serotonin metabolite in rat brains after the cessation of long-term SSRI treatment (Renoir, 2013; Stenfors and Ross, 2002; Trouvin et al., 1993). The effect develops at 48–72 h after the last injection and persists for up to 2–3 weeks (Stenfors and Ross, 2002; Trouvin et al., 1993). Our results indicate that, as in the case of adult rats, the discontinuation of neonatal FA treatment induces a long-term increase in the activity of the 5-HT system in the adolescent period, which can determine changes in rats' behaviour.

Our measurements of biogenic amines and their metabolites in the brains of 2-month-old rats have shown a reduction in the 5-HT levels in the frontal cortex and an increase in the levels of its metabolite in the hippocampus of animals neonatally exposed to FA. These results agree well with those obtained using other SSRIs. Thus, neonatal exposure to citaloptam or escitalopram led to a reduction in the 5-HT levels in the hippocampus of adult rats (Altieri et al., 2015; Schaefer et al., 2012). Furthermore, neonatal zimelidine administration increased 5-HT metabolism in the brainstem and brain cortex of adult animals (Hilakivi et al., 1995). Consequently, an imbalance in 5-HT neurotransmission may result from neonatal exposure to SSRI including FA. The imbalance may be associated with a reduction in tryptophan hydroxylase in the dorsal raphe (Maciag et al., 2006), as well as a decrease in the SERT expression in the hippocampus and frontal cortex (Maciag et al., 2006; Weaver et al., 2010) which was observed in adult animals following neonatal SSRI treatment. Our result and literature data confirm that exposure to SSRIs at an early age can disrupt the normal maturation of the serotonin system (Maciag et al., 2006). Besides the disturbances in the 5-HT system, we have registered an increase in the NE levels in the hippocampus and hypothalamus in rats exposed to FA. This means that the SERT blockade during development results in alterations in the brain NE system. It has been shown that citalopram exposure from PND 1 to PND 10 causes the locus coeruleus (LC) neuronal activity to increase in adult rats (Darling et al., 2011). In contrast, chronic SSRI exposure in adults decreases NE system activity (West et al., 2010). One of the mechanisms of the opposite effects of SSRI on these interconnected modulatory systems is the inhibitory effect of 5-HT on the LC function (Darling et al., 2011). The present results and literature data suggest that neonatal SSRI exposure alter both the 5-HT and NE systems in adults (Darling et al., 2011). The dysregulation of the monoamine systems may contribute to cognitive and emotional dysfunction (Berridge and Waterhouse, 2003).

Clinical and preclinical findings suggest long-term consequences of SSRI perinatal exposure for the offspring. However, there are no directional approaches for prevention of the consequences caused by altered 5-HT levels during early development. In the present study, we have evaluated the influence of analogue of ACTH(4–10) Semax on the effects of neonatal FA exposure. Previously, it was shown that chronic Semax administration to neonatal rats has a favourable effect on their anxiety-like behaviour and learning ability (Sebentsova et al., 2005), and also induces an increase in neurogenesis in the hippocampus (Timoshenko et al., 2009). Furthermore, the Semax administration attenuates the negative effects of chronic neonatal stress and normalizes the levels of brain-derived neurotrophic factor (BDNF) in the brain structures of rats that underwent neonatal stress (Sukhanova et al., 2018; Volodina et al., 2012). In the present study, Semax was administered from PND 15 to PND 28 to rats that had been exposed to FA during early neonatal development. It has been shown that administration of the peptide normalizes the anxiety-related behaviour in adolescent and adult rats in the EPM test under conditions that provoke the fear response. Moreover, Semax exerts a favourable effect on learning abilities impaired by the FA exposure. Also, the Semax administration restored the imbalance in monoamines systems in rats exposed to FA. The biogenic amine system plays a major role in the regulation of emotional behaviour, as well as in the learning and memory processes. Normalisation of activity of this system, caused by the Semax administration, can provide long-term effects of the drug.

There is ample evidence of a close relationship between the two major systems having effects on the brain development and neuroplasticity: the 5-HT and BDNF systems. Numerous data indicate that not only BDNF has an impact on the 5-HT-system of the brain, but 5-HT is also implicated into regulation of BDNF (Popova et al., 2017). Neonatal SSRI administration has been shown to reduce the expression of BDNF and its TrkB receptor (Boulle et al., 2016; Karpova et al., 2009). An acute BDNF intrahippocampal injection decreased extracellular levels of 5-HT and enhanced SERT function (Benmansour et al., 2008). Previously, it was reported that Semax increases the levels of BDNF and TrkB expression in cell cultures and in the brains of intact animals (Dolotov et al., 2006; Shadrina et al., 2001), as well as in the ischemic rat cortex (Dmitrieva et al., 2010). We have recently shown that Semax administration normalizes the BDNF content in the brains of adult rats underwent chronic neonatal stress (Sukhanova et al., 2018). It can be assumed that the positive effect of Semax on the behaviour and the brain biogenic amine system of animals exposed to SSRI during early development is mediated by its activating effect on the BDNF system.

6. Conclusion

Daily administration of antidepressant FA to animals from postnatal days 1 to 14 leads to long-term disturbances in their anxiety-like behaviour and learning abilities, which is probably associated with an imbalance of the brain biogenic amine system. The Semax administration after discontinuation of fluvoxamine treatment largely compensates for the disturbances caused by the neonatal SERT blockade. In animals that received Semax injections, the anxiety-related behaviour is normalized and the impairment of cognitive functions is attenuated. The protective effects of Semax on the behaviour of rats that were neonatally exposed to fluvoxamine are related to the normalisation of the brain biogenic amine system activity.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.npep.2020.102114.

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