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Behavioral and Neurochemical Characteristics of Two Months Old WAG/Rij Rats with Genetic Absence Epilepsy

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Abstract WAG/Rij rats are genetic animal model of absence epilepsy with comorbidity of depression. The first spike-wave discharges (SWDs) in WAG/Rij rats begin to appear at the age of 2-3 months and are fully manifested by 5-6 months. Occurrence of SWDs in the EEG is the main index of absence epilepsy. Previously it has been shown that the extensive absence epilepsy in 5-6 months old WAG/Rij rats is accompanied by decrease of dopamine and its metabolites concentrations in the meso-cortico-limbic and nigro-striatal dopaminergic brain systems, resulting in the expression of depression-like behavioral symptoms, and impairments of the learning and memory processes. In 36 days old WAG/Rij rats, SWDs are not manifested, deficiency of the mesolimbic dopamine is not revealed, and symptoms of depression-like behavior are not expressed. In this study, behavior in the open field, light-dark choice, forced swimming tests, monoamines and their metabolites concentrations in 5 brain structures (prefrontal cortex, nucleus accumbens, hypothalamus, striatum, hippocampus) were investigated in two months old WAG/Rij rats in comparison with age-matched Wistar rats. Reduced concentration of the dopamine and its metabolites, and increased concentration of the serotonin was found in WAG/Rij rats compared with Wistar rats only in the prefrontal cortex, indicating that the prefrontal cortex is the brain structure where neurochemical abnormalities appear first. No substantial changes in the monoamine and their metabolites concentrations have been revealed in other brain structures. Two months old WAG/Rij rats didn't exhibit depression-like behavior in the forced swimming test, and learning/memory deficits in the passive avoidance test, but they showed behavioral changes, indicating increase anxiety/stress-reactivity, and alterations in learning/memory in the active avoidance test. Results suggest that two month-old WAG/Rij rats are at the stage of so called "pre-pathology" (increased anxiety and stress reactivity) preceding the development of depression-like behavior and substantial cognitive impairments which are co-morbid to fully expressed absence epilepsy in 5-6 months old rats of this strain.

Keywords: *2 months old WAG/Rij rats, monoamines, dopamine, anxiety, depression-like behavior, learning and memory*

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1. Introduction

The WAG/Rij strain is a genetic animal model of human absence epilepsy [7,41]. The principal difference between absence epilepsy and convulsive epilepsy is a profile of epileptic discharge. The absence spike – wave discharge (SWD) consists of an inhibitory phase and action potential. The inhibitory phase is reflected in the EEG as a slow wave, while a spike discharge represents excitation of cells (action potential). Rebound spike appears at the end of the inhibitory period and the repeated cycles occur again. SWD is generated by hyperpolarization activated cyclic nucleotide gated pacing channel (I_h channel), which is localized in the pyramidal neurons of the III - V layer of the somatosensory cortex and thalamic nucleus [7].

The SWDs in WAG/Rij rats begin to appear at the age of 2-3 months and get full manifestation at 5-6 months [41]. The four subunits HCN1 – HCN4 form the homomeric or heteromeric $\boldsymbol{I_h}$ channel. Enhancement of SWDs is associated with reduction of a number of HCN1 subunit in the channel composition [7]. In WAG/Rij rats at the age of 5-6 months the enhancement of SWDs is accompanied by decrease of meso-cortico-limbic and nigro-striatal dopaminergic (DA) systems. The deficiency of these systems leads to depression-like behavior and disturbances of learning and memory processes [7,41]. The impairments of meso-cortico-limbic and nigro-striatal DA systems in symptomatic WAG/Rij rats produce a

significant decrease of DA and its metabolites in five brain regions: in the prefrontal cortex, nucleus accumbens, striatum, hypothalamus and hippocampus [42,43]. The DA deficit in WAG/Rij rats leads to decreases in D1-like DA receptors density in the nucleus accumbens core and head of the caudate nucleus [7], and increases in D2-like DA receptors density in the motor, sensorimotor, parietal cortex [7], and in the core of the nucleus accumbens [42]. Interestingly, that in the 36-days old WAG/Rij rats when SWDs are not developed yet, no DA deficit in the meso-cortico-limbic brain system and no symptoms of depression-like behavior were seen [43].

The aim of this work was to investigate the levels of monoamines and its metabolites in the meso-cortico-limbic and nigro-striatal brain regions in 2 months old WAG/Rij and Wistar rats in combination with a study of behavior in the light-dark choice, open field, forced swimming tests, and learning and memory in passive and active avoidance tests. Taking into account that behavioral and cognitive impairments in WAG/Rij rats are dopamine-dependent [7,39,41] we also investigated behavioral and neurochemical effects of a specific 'loading' – acute injection of DA precursor L-DOPA (Madopar) in 2 months old WAG/Rij and Wistar rats.

2. Materials and Methods

2.1. Animals

Male inbred WAG/Rij rats and age-matched outbred Wistar rats were used in this study. WAG/Rij and Wistar rats were born and raised at the Institute of Higher Nervous Activity and Neurophysiology Russian Academy of Sciences. The WAG/Rij rats represented approximately the 30-31th generations from parents originally obtained from Radboud University Nijmegen, The Netherlands. The parents of Wistar rats were purchased from breeding company ''Stolbovaya'' and then were housed at the Institute of Higher Nervous Activity. At the age of 2 months WAG/Rij and Wistar rats weighed approximately 100 – 120 g. All rats were housed under a natural light–dark regime (about 10 h of daytime light). Animals were housed in standard plastic cages in groups of 3–4 animals per cage. Food and tap water were available ad lib except the periods of the behavioral testing. Experiments were performed in accordance with the Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm).

2.2. Drugs

WAG/Rij and Wistar rats were divided into two groups - control and experimental. The experimental groups of WAG/Rij and Wistar rats received a single intraperitoneal Madopar (F. Hoffmann-La Roche Ltd, Basel, Switzerland) injection. Madopar (containing of DA precursor levodopa + peripheral decarboxylase inhibitor - benserazide, one pack of medopar contains 100 mg of levodopa + 25 mg of benserazide) was injected in dose of 25 mg/kg of levodopa in a volume of 250 µl. The control groups received 250 µl of vehicle. The concentration of monoamines and their metabolites were determined 1.5 hours after administration of madopar and on the second day after injection.

2.3. Series of the Experiments

Two series of experiments have been carried out in this study. In the first series of experiments, 2 months old WAG/Rij and Wistar rats were examined 1.5 h after administration of Madopar and on the second day after injection in tests assessing depression and anxiety. In the second series of experiments, 2 months old WAG/Rij and Wistar rats were examined 1.5 h after administration of Madopar and on the second day after injection in tests assessing learning and memory.

2.4. Behavioral Tests

To detect differences in behavior between WAG/Rij and Wistar rats we used the conventional tests for assessment of anxiety (open field, light–dark choice tests) and depression (forced swimming test). Behavior of animals was tested at the age of 2 months when the WAG/Rij rats start to show the symptoms of absence-epilepsy [41]. Animal's behavior was recorded and analyzed using software and hardware complex 'EthoVision XT. Version 3.1' (Noldus Information Technology).

2.4.1. Open Field Test

The apparatus represented a circular arena, 100 cm in diameter with a 30 cm wall, and with a floor divided into 32 squares by five vertical and five horizontal lines. Four squares were taken as the ''center'' of the field. The test room was dimly lit (40 W) in order to decrease the stressfulness of the test. An animal was placed in the center of the field (80 lx), and the following variables were recorded for 10 min: time to leave the center (s), the number of squares crossed (horizontal activity), rearing (vertical activity), grooming, and fecal boli (defecation) [37]. The open field was cleaned each time after removing a rat from arena. A low ambulatory activity and a short amount of time spent in the center of the open field are commonly interpreted as high level of anxiety and vice versa [16,32,37].

2.4.2. Light–dark Choice Test

The apparatus consisted of two compartments with an opening between them. The large (36 x 18 cm) compartment was light (100 lx) and the small one (18 x 17 cm) was dark (<5 lx). A rat was placed in the light compartment facing away from the opening, and the following behavioral parameters were measured for 5 min: latency of entering the dark compartment, the time spent in each compartment, the number of transitions between compartments, the number of rearing in the light compartment, and the number of ''risk assessments'' (aborted attempts to entry into the light compartment) [37]. A short time spent in the light compartment, a low number of transitions between compartments, and a large number of risk assessments – aborted attempts and/or partial entries to the lit compartment (ethologically derived measure) is considered as a high level of anxiety in the test and vice versa [10,12,18,20,38,46].

2.4.3. Forced Swimming Test

The apparatus was a cylinder (height 47 cm, inside diameter 38 cm) containing 38 cm of tap water maintained

at 22 ± 1°C. The procedure used in this study corresponded to that described by Porsolt [31], with some minor modification [34,35,36,37,40] It has been shown that using this modification of the Porsol't forced swimming test the depressive-like features of behavior and the antidepressant potential of the drugs are well-reproducible, and a possible interference of memory processes is avoided. Briefly, rats were individually forced to swim for 5 min. The duration of passive swimming (immobility), the duration of the first episode of active swimming, the duration of swimming, and the number of dives and boli were measured. The criterion for passive swimming or immobility was floating vertically in the water, making only those movements necessary to keep the head above the surface of the water. The first episode of active swimming represented more vigorous activity than swimming: strong movements of all four limbs, jumping, the front limbs breaking the surface of the water that resemble climbing or scratching the wall [13,25]. Increased immobility and decreased active behaviors in this test is considered as depressive-like behavior [25,35,37,38,40].

2.4.4. Passive Avoidance Learning

A two compartment passive avoidance apparatus was used. A rat was placed into the light compartment of the test box and the time spent in it was measured. When animal moved into the dark compartment, the guillotine door was closed and immediately electric foot shock (0.5 mA) was applied for 3 sec. The passive avoidance response was tested on the second day by placing the animal in the light compartment and recording the latency of entering the dark compartment. Monitoring was limited to 300 seconds.

2.4.5. Active Avoidance Learning

The defensive conditioned reflex of two-way avoidance was established in a shuttle-box. Flickering light served as a conditional stimulus. 5 seconds later an electric foot shock (0.5 mA for 3 sec) was applied to the grid floor. The combined action of the light and foot shock went on until the animal moved into the opposite, "safe" compartment. If the rat didn't cross to the opposite compartment, it would receive a foot shock. When the rat crossed to the opposite compartment during the conditioned stimulus, an avoidance response was recorded. The interval between consecutive trials was 30 s. The animals learned to avoid a foot shock during a single training session on days 1 and 2. The training sessions consisted of 50 consecutive trials. The number of avoidance responses was recorded as a measure of learning.

The results of the behavioral tests were expressed as the mean ± the standard error of the mean. For statistical analysis, we used the Mann-Whitney U test. The differences were considered as significant at $p < 0.05$.

2.5. Neurochemical Studies

The concentrations of monoamines and their metabolites in different brain regions of the rats were determined by high-performance liquid chromatography with electrochemical detection (HPLC). The animals were decapitated under ether anesthesia 1.5 hours after administration of madopar the 1st series of experiments) or on the second day after injection (the 2nd series of experiments). In two series of experiments were studied, 20 rats of WAG/Rij strain and 20 Wistar rats strain, the 10 rats of WAG/Rij and the 10 rats of Wistar strain with vehicle administration in each series and 10 rats of WAG/Rij and 10 rats of Wistar strain with madopar administration in each series. The appropriate brain regions from each animal (prefrontal cortex, nucleus accumbens, hypothalamus, hippocampus, and striatum) were removed rapidly, placed in liquid nitrogen, weighed, and stored at -80 C until chromatographic studies were performed. The isolated brain structures were homogenized at +4°C in a glass homogenizer with a Teflon pestle (0.2 mm) at 3000 rpm. The extraction and homogenization medium was 0.1 N $HClO_4$ with an internal standard of 0.5 nmole/mL DOBA (3,4-dioxybenzylamine), a catecholamine that is never present in native tissue. The nucleus accumbens was homogenized in 40 volumes of the medium and other brain structures were homogenized in 20 volumes. The samples were centrifuged at +4°C and 10000 *g* for 15 min. The supernatant was used for measurements of monoamines and their metabolites.

LC – 304T chromatograph (BAS, West Lafayette, USA) with a Rheodyne 7125 injector and 20 мL loop was used. The substances studied were separated in a 4 x 100 mm ReproSil–Pur ODS–3.4 x 100 mm, 3 m reversed–phase column, (Dr. Majsch GmbH, Germany). We used a PM–80 pump (BAS, West Lafayette, USA), the elution rate of the mobile phase was 1.0 mL/min at a pressure of 200 atm. The mobile phase was 0.1 M citrate–phosphate buffer that contained 1.1. mMoctanesulfonic acid, 0.1 mM EDTA, and 9% acetonitrile (pH = 3.0). The flow rate was 1 mL/min. Measurements were performed using a LC–4B electrochemical detector (BAS, West Lafayette, USA) with a glass–carbon electrode (+0.85 V) and an Ag/AgCl reference electrode. Recording of samples was performed using the MULTICHROM 1.5 hardware–software complex (AMPERSEND). To calibrate the chromatograph, we used mixtures of measured substances at a concentration of 500 pmol/mL. The concentrations of monoamines in the experimental samples were calculated using the method of an "internal standard" taking the ratios between the areas in a standard mixture and sample into account. We measured the contents of noradrenaline (NA), dopamine (DA) and its metabolites, 3,4–dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5–HT) and its metabolite 5–hydroxyindoleacetic acid (5–HIAA).

The results of the neurochemical measurements were expressed as the mean ± the standard error of the mean (M±.S.E.M). To identify significant differences between the exposed and control animals, the one - way analysis of variance (ANOVA) was used. An a posteriori comparison of means was carried out according to the criterion of the least significant difference LSD (ANOVA). To determine the general direction of the shift in the concentration of the substance after the exposure, the nonparametric Z - sign test was used. The differences were considered significant at $p < 0.05$. The p values from 0.05 to 0.1 were considered as an expression of a trend.

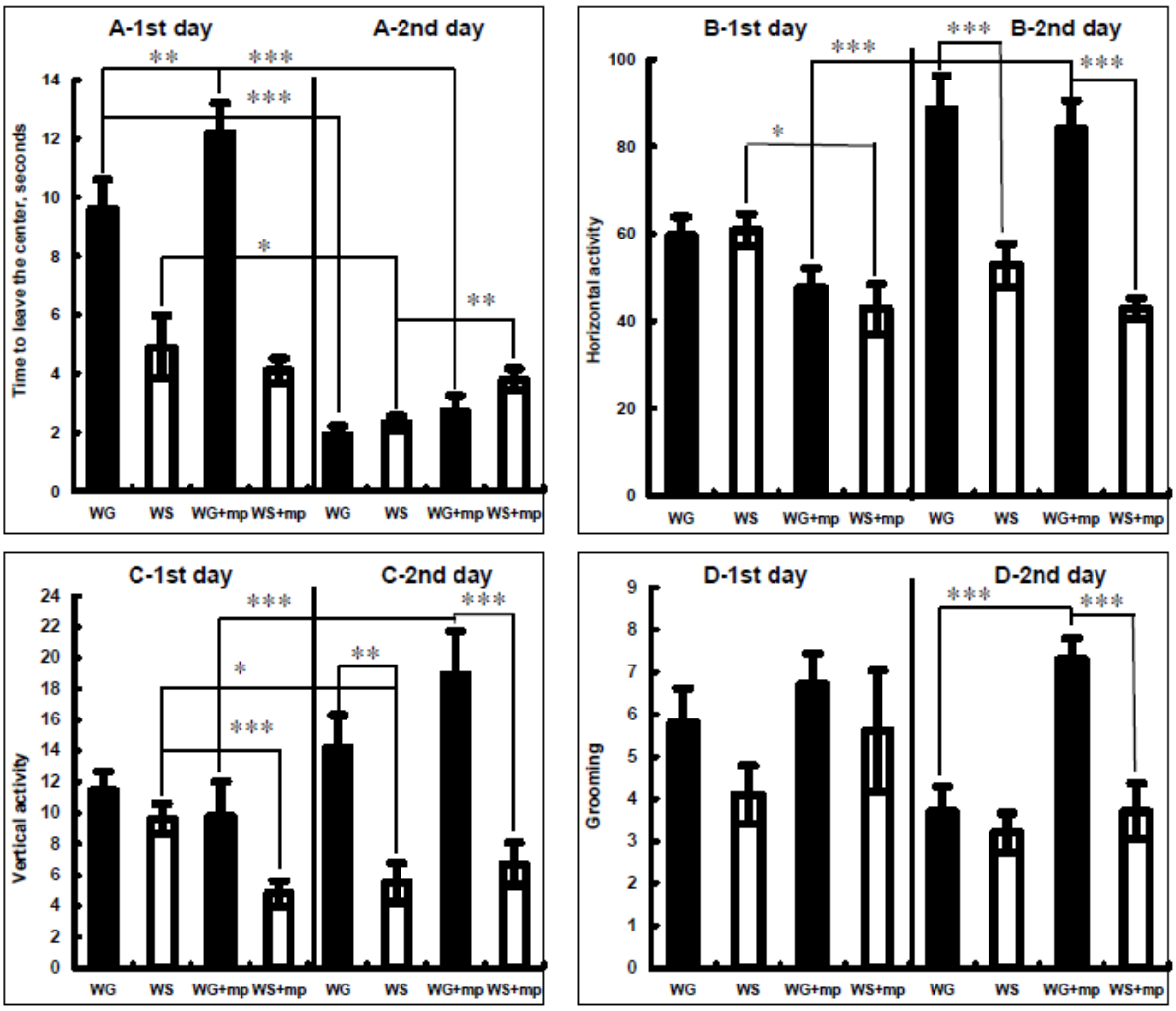

Figure 1. Open field test. (WR), WAG/Rij + vehicle (n=10); (WS), Wistar + vehicle (n=10); (WG + mp), WAG/Rij + madopar (n=10) ; (WS + mp), Wistar + madopar (n=10). (A), Time to leave the center, seconds. (B), Horizontal activity - the number of squares crossed. (C), Vertical activity - the number of rearing. (D), Grooming - the number of washings. * - $p < 0,05$; ** - $p < 0,01$; *** - $p < 0,001$

3. Results

Open field test. Vehicle-treated WAG/Rij rats at the age of 2 months did not differ from their Wistar counterparts by the time to leave the center of the open field, the horizontal and vertical activities, and grooming reactions (Figure 1). The madopar injection significantly increased the time to leave the center of arena in WAG/Rij rats, but had no effect in Wistar rats (Figure 1, A). There was a significant difference in latency to leave the center of arena between the first and second days in both strains subjected to injections of vehicle and madopar, but no difference was found between the WAG/Rij and Wistar rats independently of what substance (madopar or vehicle) was injected. The latency to leave the center of arena on the second day was substantially less than on the first day of testing in both groups of rats, possibly due to habituation to the experimental situation and reduction of anxiety and fear (Figure 1, A).

Madopar injection affected locomotor activity both on the first and second days, with statistically significant difference between WAG/Rij and Wistar rats on the second day (Figure 1, B, C). The compound decreased the horizontal and vertical activities in Wistar rats, but not in WAG/Rij rats. On the second day after injection, the horizontal and vertical activities were higher in WAG/Rij rats compared to Wistar counterparts.

Madopar significantly increased grooming in WAG/Rij rats but only on the second day after its administration compared to vehicle-treated WAG/Rij rats and madopar- and vehicle-treated Wistar rats (Figure 1, D).

Light–dark choice test. In this paradigm, 5 of 6 parameters tested initially did not differ in WAG/Rij rats compared with Wistar rats (Figure 2). However, the number of "risk assessments" in WAG/Rij rats was significantly higher than in Wistar controls (Figure 2, E). On the second day, the latency of entering into the dark compartment compared to the first day substantially decreased in Wistar rats (Figure 2, A). This might be a result of habituation to the experimental situation and reduction of anxiety. Madopar administration significantly reduced the latency of entering into the dark compartment in Wistar rats on the second day, which could also reflect a habituation to the context and reduction of anxiety (Figure 2, A). Madopar decreased the time spent in the light compartment (Figure 2, B) and, consequently, increased the time spent in the dark compartment only in WAG/Rij rats (Figure 2, C). This outcome may be interpreted as increases in anxiety level in WAG/Rij rats, but not in Wistar controls, due to madopar injection. Madopar decreased the number of transitions between the

light and dark compartments both in WAG/Rij and Wistar rats (Figure 2, D). This result shows an increase in anxiety in both groups of rats after madopar administration. On the second day after madopar administration, the number of transitions between compartments in both groups recovered to the basal level, effect was statistically significant for WAG/Rij rats only. A similar effect was seen for vertical activity (rearing) in the light-dark choice test in WAG/Rij rats (Figure 2, F).

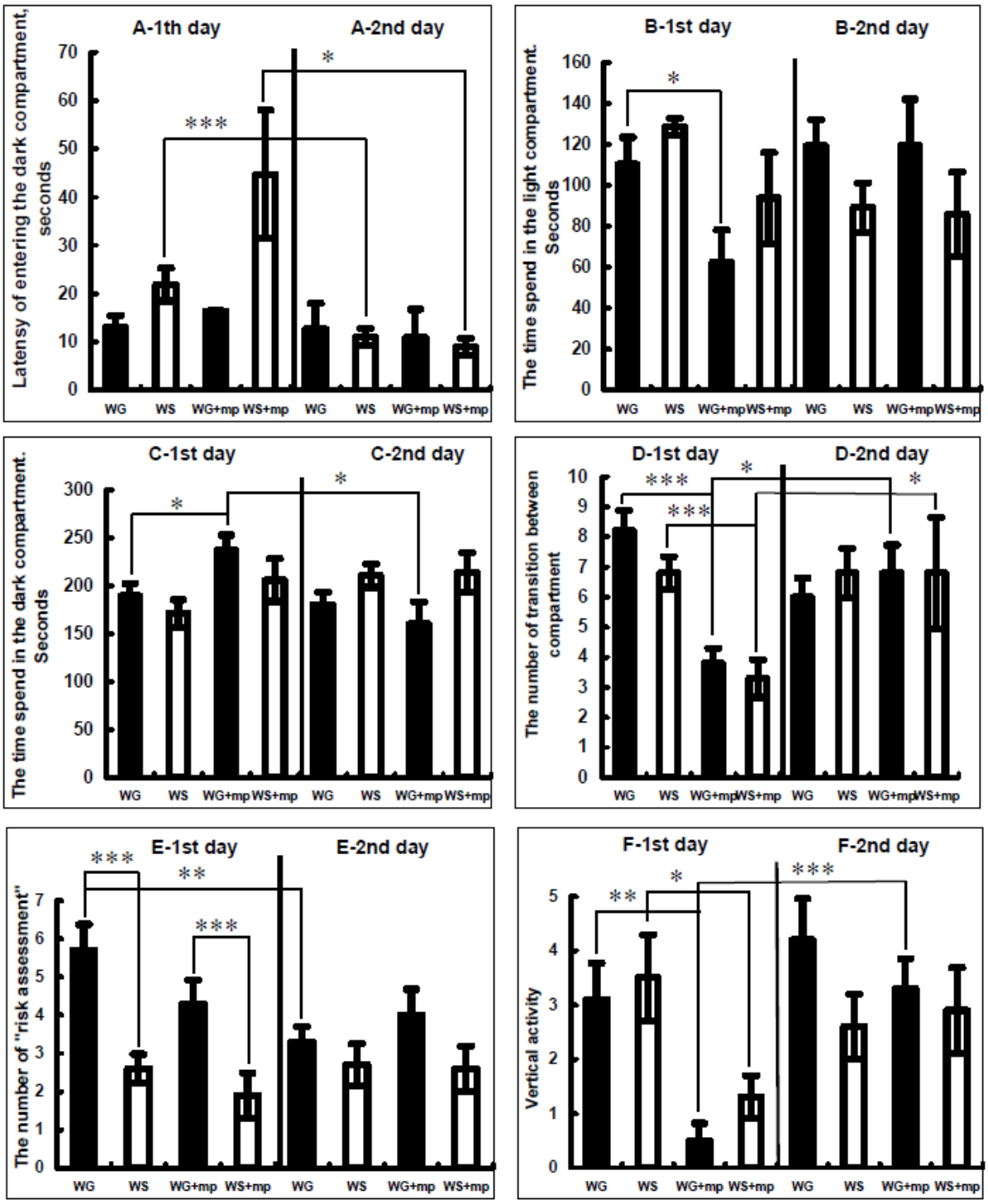

Figure 2. Light-dark choice test. (A), Latency of entering the dark compartment, seconds. (B), the time spent in the light compartment, seconds. (C), the time spent in the dark compartment, seconds. (D), the number of transitions between compartments. (E), the number of "risk assessments". (F), Vertical activity - the number of rearing. * - $p < 0,05$; ** - $p < 0,01$; *** - $p < 0,001$. Other designations as in Figure 1

Forced swimming test. The results of the forced swimming test are shown in Figure 3. The data on duration of swimming and number of dives are not presented here because differences of these parameters were insignificant between the rats' strains and madopar/vehicle-treated groups. There was no difference between WAG/Rij and Wistar rats at the age of 2 months in the latency of the first episode of active swimming. Madopar significantly increased this behavioral measure in WAG/Rij rats compared to their Wistar counterparts (Figure 3, A). In the vehicle-treated WAG/Rij rats, the duration of the first episode of active swimming was significantly shorter than in Wistar rats (Figure 3B). Madopar reduced the duration of the first episode of active

swimming in Wistar rats, but not in WAG/Rij rats (Figure 3B). Although madopar did not change substantially the first episode of active swimming in WAG/Rij rats, it markedly enhanced swimming movements ("climbing", jumping). There was no difference in immobility time between vehicle-treated WAG/Rij and Wistar rats. However, madopar administration significantly reduced the immobility time only in WAG/Rij rats compared to Wistar rats (Figure 3, C), indicating antidepressant-like effect of the compound.

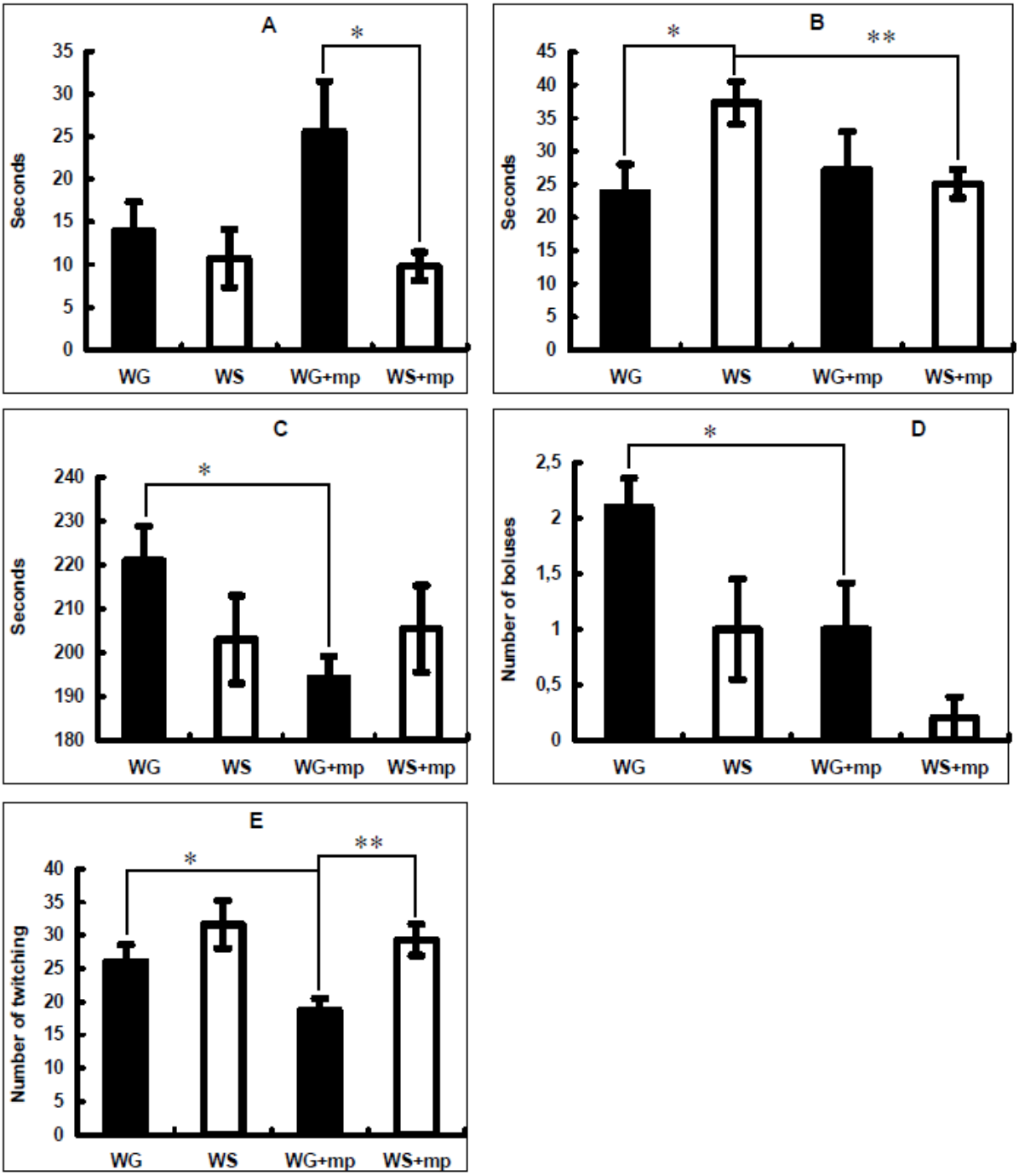

Figure 3. Forced swimming test. (A), latency to start swimming after placement in the water, seconds. (B), the duration of the first episode of active swimming ('climbing'), seconds. (C), the duration of immobility, seconds. (D), the number of boluses. (E), the number of head twitching, * - $p < 0,05$; ** - $p < 0,01$. Other designations as in Figure 1

Madopar reduced the number of boli in WAG/Rij rats (Figure 3, D).

Two-month old vehicle-treated WAG/Rij and Wistar rats initially did not differ from each other on head twitches. Madopar administration significantly reduced the number of head twitches only in WAG/Rij rats compared to both vehicle-treated WAG/Rij rats and madopar-treated Wistar rats (Figure 3, E).

Passive avoidance learning. The vehicle- and madopar-treated WAG/Rij and Wistar rats equally learned a passive avoidance task. The latency of entry into the dark compartment in WAG/Raj and Wistar rats did not differ on the first day. Madopar administration did not affect the latency (Figure 4). On the second day the latency of entry into the dark compartment substantially increased reflecting the learning process, no differences were found between groups. On the second day the rats retained a fear

of entry into the dark compartment where a day before they received a foot shock. Learning of passive avoidance task were similar in WAG/Rij and Wistar rats both under vehicle and madopar conditions (Figure 4).Thus, there was no learning and memory deficit in 2-month old WAG/Rij rats relative to Wistar rats.

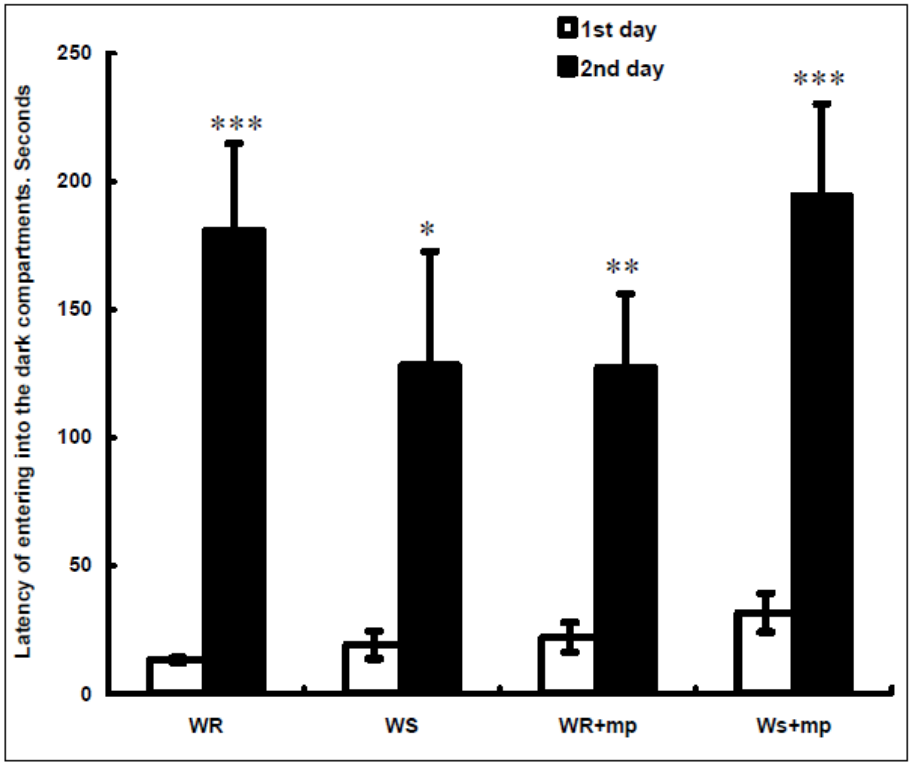

Figure 4. Passive avoidance conditioning. The 2nd day vs. the 1st day: * - p < 0,05; ** - p < 0,01; *** - p < 0,001. Other designations as in Figure 1

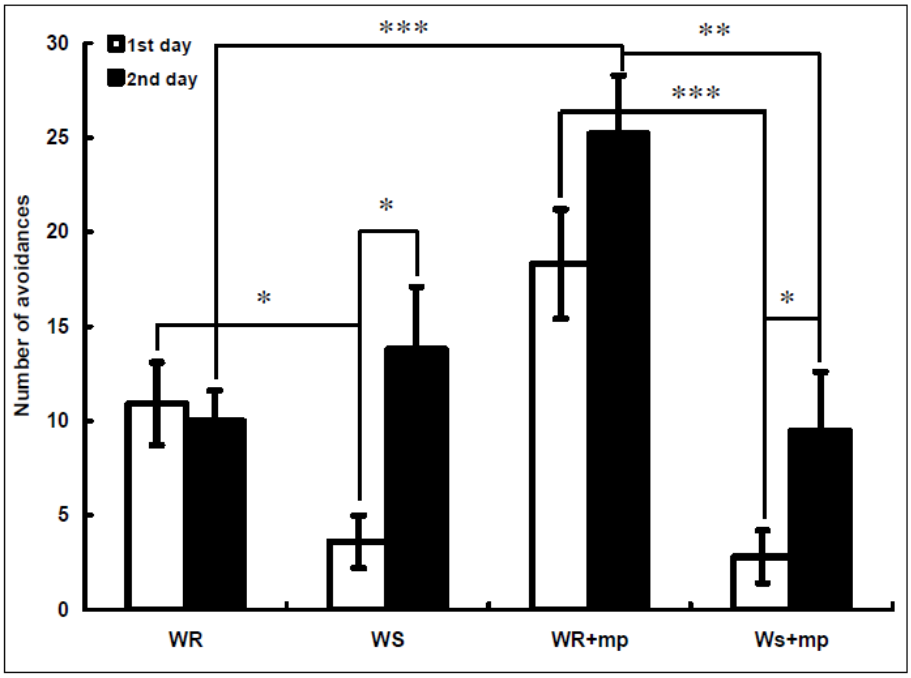

Figure 5. Active avoidance conditioning. * - p < 0,05; ** - p < 0,01; *** - p < 0,001. Other designations as in Figure 1

Active avoidance learning. The active avoidance conditioning paradigm appeared to be more sensitive test to detect differences between 2 months old WAG/Rij and Wistar rats than passive avoidance conditioning (Figure 5). On the first day, the WAG/Rij rats learned the task much better than Wistar rats. On the second day, WAG/Rij rats showed deficit in learning and memory. In WAG/Rij rats the numbers of avoidances on the first and second day were approximately the same, while in Wistar controls the number of avoidance responses significantly increased on the second day compared with the first day. The calculation of the memory trace storage showed that I_{MT} in WAG/Rij rats were -8.26%, i.e. the number of avoidance reactions on the second day was less than the number of active avoidance reactions on the first day (8.16%). Wistar rats showed much more active avoidances on the second day than on the first day. The calculation of the memory trace storage showed that I_{MT} in Wistar rats

were 283.33%. Madopar exerted different effects on learning in WAG/Rij and Wistar rats. On the first day of training, madopar enhanced learning in WAG/Rij rats compared to vehicle-treated WAG/Rij rats (Figure 5). Madopar increased the number of avoidances relative to control WAG/Rij rats on the first and second days. The compound removed a memory deficit revealed in vehicle-treated WAG/Rij rats. The number of avoidances on the second day was much higher than the number of avoidances on the first day in madopar-treated WAG/Rij rats. The calculation of the memory trace storage showed that I_{MT} in WAG/Rij rats subjected to madopar treatment is 37.71%. The compound had a little effect on learning and memory in Wistar rats. The calculation of the memory trace storage shows that I_{MT} in Wistar rats under madopar were 239.29%. This finding indicates a higher sensitivity to the compound of 2-month old WAG/Rij rats compared with age-matched Wistar controls.

The monoamines and their metabolites concentration in the brain structures in 2-month old WAG/Rij and Wistar rats.

The first series of experiments.

Neurochemical studies have revealed the significant differences between 2-month old WAG/Rij and Wistar rats, but only in the prefrontal cortex. There was a statistically significant decrease of DA concentration and its metabolite HVA, and an increase of 5-HT and decrease of its metabolite 5-HIAA in WAG/Rij rats compared with Wistar controls. The DOPAC concentration in WAG/Rij rats was less than that in Wistar rats, with a tendency to a significance (p = 0.059) (Table 1). In all other brain structures there were no statistically significant differences in the concentration of monoamines and their metabolites between 2-month old WAG/Rij and Wistar rats (Table 1). Reduced activity of the DA-ergic system in the prefrontal cortex may reflect a state of heightened anxiety [15] and stress-reactivity [23] in 2 months old WAG/Rij rats.

Table 1. The monoamines and their metabolites concentration (nmol/g wet tissue) in the brain structures of 2-month-old WAG/Rij and Wistar rats 1.5 hours after administration of vehicle and madopar 0,25 mg/kg

	Vehicle		Madopar		P values			
	WAG/Rij (n = 5)	Wistar (n = 5)	WAG/Rij (n = 5)	Wistar (n = 5)	p_t =	p_t =	p_t =	p_t =
Groups	1	2	3	4	1 vs. 2	1 vs. 3	2 vs. 4	3 vs. 4
Prefrontal cortex								
NA	0,5 ± 0,08	0,37 ± 0,04	9,43 ± 1,54	14,48 ± 4,83	0,223	**0,001**	**0,031**	0,399
DOPAC	0,07 ± 0,02	0,29 ± 0,08	2,24 ± 0,28	2,8 ± 0,73	***0,059***	**0,0001**	**0,01**	0,541
DA	0,3 ± 0,02	0,39 ± 0,027	8,62 ± 0,58	6,76 ± 1,68	**0,05**	**0,0001**	**0,009**	0,378
HVA	0,06 ± 0,01	0,16 ± 0,032	2,87 ± 0,36	3,16 ± 0,75	**0,006**	**0,0001**	**0,007**	0,574
5-HIAA	0,92 ± 0,03	1,1 ± 0,032	1,12 ± 0,08	1,21 ± 0,12	**0,036**	***0,066***	0,440	0,761
5-HT	3,45 ± 0,08	2,94 ± 0,076	2,31 ± 0,03	2,11 ± 0,17	**0,003**	**0,0001**	**0,004**	0,346
Nucleus accumdens								
NA	2,93 ± 0,32	2,53 ± 0,2	2,12 ± 0,34	1,94 ± 0,31	0,381	0,165	0,732	0,561
DOPAC	3,58 ± 0,35	3,47 ± 0,28	6,24 ± 0,78	10,27 ± 3,75	0,387	**0,0004**	**0,029**	0,531
DA	27,9 ± 2,89	25,22 ± 1,88	24,01 ± 1,58	25,44 ± 5,06	0,808	0,586	0,424	0,798
HVA	1,97 ± 0,24	2,05 ± 0,07	5,43 ± 0,73	8,8 ± 2,96	0,109	**0,008**	**0,041**	0.221
3-MT	1.97 ± 0.24	2.05 ± 0.07	5.43 ± 0.73	8.8 ± 2.96	0.152	**0.0004**	**0,018**	0,804
5-HIAA	2,2 ± 0,18	2,05 ± 0,11	2,02 ± 0,15	2,11 ± 0,23	0,734	0,469	0,228	0,477
5-HT	6,01 ± 0,63	4,93 ± 0,31	4,03 ± 0,44	3,76 ± 0,2	0,111	0,141	0,210	0,308
Hypothalamus								
NA	4,49 ± 0,29	4,26 ± 0,23	3,72 ± 0,16	3,42 ± 0,4	0,597	0,826	0,114	0,826
DOPAC	0,18 ± 0,02	0,19 ± 0,03	5,00 ± 2,50	1,94 ± 0,5	0,216	**0,0004**	**0,027**	0,313
DA	1,29 ± 0,1	1,19 ± 0,14	4,03 ± 0,93	2,45 ± 0,53	0,962	**0,001**	**0,005**	0,601
HVA	0,14 ± 0,02	0,13 ± 0,02	4,51 ± 1,46	2,21 ± 0,49	0,846	**0,0004**	**0,009**	0,817
5-HIAA	1,62 ± 0,11	1,57 ± 0,1	1,51 ± 0,1	1,51 ± 0,08	0,337	0,480	***0,92***	0,765
5-HT	4,07 ± 0,32	3,68 ± 0,2	2,62 ± 0,07	3,09 ± 0,26	0,654	0,112	**0,03**	0,273
Striatum								
NA	0,63±0,33	0,35±0,27	2,26±0,51	2,54±1,88	0,568	1,00	0,145	0,505
DOPAC	5,02 ± 0,25	4,74 ± 0,24	9,53 ± 1,48	15,57 ± 4,91	0,485	**0,0004**	**0,029**	0,798
DA	53,68 ± 2,51	50,46 ± 3,06	57,19 ± 3,07	66,86 ± 10,45	0,584	**0,0004**	***0,085***	0,901
HVA	4,14 ± 0,21	3,9 ± 0,22	8,65 ± 1,12	13,19 ± 3,16	0,563	**0,0004**	**0,016**	0,599
3-MT	0.56 ± 0.02	0.52 ± 0.01	0.46 ± 0.06	0.34 ± 0.06	0.546	***0.06***	**0.012**	0.691
5-HIAA	2,67 ± 0,17	2,73 ± 0,09	2,84 ± 0,12	3,09 ± 0,35	0,117	**0,006**	0,165	0,367
5-HT	3,87 ± 0,26	3,7 ± 0,14	3,46 ± 0,22	3,39 ± 0,15	0,361	0,816	0,307	0,947
Hippocampus								
NA	1,04 ± 0,07	1,15 ± 0,04	1,12 ± 0,007	1,12 ± 0,02	0,233	0,352	0,518	0,84
DOPAC	0,07 ± 0,04	0,05 ± 0,004	0,99 ± 0,15	1,54 ± 0,56	0,35	**0,001**	**0,033**	0,445
DA	0,11 ± 0,01	0,12 ± 0,03	1,27 ± 0,15	1,54 ± 0,5	0,78	**0,0004**	**0,027**	0,676
HVA	–	–	1,37 ± 0,17	1,47 ± 0,48	–	–	–	0,873
5-HIAA	0,87 ± 0,17	0,97 ± 0,04	1,23 ± 0,04	1,52 ± 0,1	0,277	0,136	***0,051***	0,057
5-HT	1,68 ± 0,06	1,53 ± 0,09	1,45 ± 0,07	1,53 ± 0,14	0,929	***0,083***	0,502	0,673

Note: values are the mean ± SEM. Statistically significant differences are shown in bold and underlined, a tendency is shown in Italic.

Table 2. The monoamines and their metabolites concentration (nmol/g wet tissue) in the brain structures of 2-month-old WAG/Rij and Wistar rats on the second day after administration of vehicle and madopar 0,25 mg/kg

	Vehicle		Madopar		P values			
	WAG/Rij (n = 5)	Wistar (n = 5)	WAG/Rij (n = 5)	Wistar (n = 5)	p_t =	p_t =	p_t =	p_t =
Groups	1	2	3	4	1 vs. 2	1 vs. 3	2 vs. 4	3 vs.4
Prefrontal cortex								
NA	1,33±0,05	1,06±0,04	1,4±0,16	1,06±0,06	**0,004**	0,696	0,975	0,109
DOPAC	0,19±0,02	0,27±0,02	0,15±0,01	0,23±0,04	**0,038**	0,136	0,4110	0,129
DA	0,28±0,06	0,26±0,03	0,31±0,05	0,31±0,05	0,791	0,769	0,510	0,993
HVA	0,06±0,01	0,15±0,01	0,07±0,01	0,12±0,02	**0,003**	0,554	0,279	0,115
3-MT	0,04±0,02	0,09±0,05	0,03±0,01	0,08±0,05	0,418	0,616	0,909	0,430
5-HIAA	0,57±0,01	0,83±0,06	0,72±0,04	0,59±0,07	**0,004**	**0,022**	**0,044**	0,195
5-HT	2,60±0,11	2,59±0,15	2,62±0,90	2,49±0,16	0,975	0,901	0,699	0,577
Nucleus accumdens								
NA	2,53±0,29	2,35±0,37	2,32±0,46	2,99±0,59	0,737	0,738	0,463	0,448
DOPAC	5,12±0,15	4,62±0,62	5,02±0,40	4,99±0,37	0,509	0,845	0,661	0,961
DA	42,47±1,78	41,54±5,31	46,86±3,03	44,67±2,74	0,886	0,295	0,652	0,644
HVA	2,33±0,15	2,55±0,30	2,30±0,15	2,61±0,12	0,579	0,907	0,860	0,183
3-MT	0,33±0,03	0,27±0,05	0,40±0,08	0,29±0,12	0,378	0,458	0,706	0,294
5-HIAA	3,13±0,15	3,29±0,24	3,88±0,44	3,42±0,34	0,645	0,184	0,781	0,472
5-HT	8,15±0,52	7,34±0,63	8,01±0,31	8,33±1,10	0,400	0,838	0,476	0,791
Hypothalamus								
NA	5,43±0,23	4,79±0,13	5,06±0,15	4,85±0,10	***0,065***	0,539	0,778	0,319
DOPAC	0,27±0,02	0,24±0,01	0,2±0,02	0,28±0,02	0,224	**0,036**	0,14	**0,025**
DA	1,55±0,24	1,51±0,10	1,33±0,14	1,42±0,09	0,906	0,490	0,536	0,627
HVA	0,11±0,02	0,20±0,05	0,11±0,03	0,12±0,01	0,125	0,954	0,160	0,818
3-MT	0,05±0,03	0,03±0,01	0,06±0,02	0,04±0,01	0,570	0,579	0,434	0,479
5-HIAA	1,82±0,14	1,73±0,10	2,15±0,15	1,93±0,17	0,664	0,183	0,391	0,406
5-HT	3,67±0,14	3,86±0,16	4,03±0,06	4,15±0,17	0,457	0,072	0,301	0,550
Striatum								
NA	0,53±0,04	0,71±0,25	0,55±0,07	0,41±0,10	0,655	0,899	0,340	0,331
DOPAC	3,51±0,2	4,60±0,40	3,05±0,12	4,50±0,75	***0,069***	0,148	0,920	0,126
DA	44,21±3,78	50,39±3,41	36,53±2,40	51,79±8,87	0,309	0,163	0,898	0,176
HVA	2,94±0,17	4,44±0,32	2,86±0,12	4,09±0,71	**0,006**	0,752	0,700	0,165
3-MT	0,41±0,05	0,53±0,09	0,44±0,09	0,57±0,12	0,311	0,828	0,816	0,463
5-HIAA	2,01±0,13	2,9±0,31	2,84±0,22	2,63±0,48	**0,044**	**0,02**	0,685	0,729
5-HT	3,9±0,33	4,35±0,47	3,85±0,14	3,89±0,69	0,503	0,905	0,637	0,956
Hippocampus								
NA	2,07±0,34	1,40±0,07	1,75±0,11	1,32±0,09	0,072	0,264	0,597	**0,021**
DOPAC	0,09±0,01	0,07±0,47	0,08±0,01	0,11±0,02	0,474	0,477	0,284	0,265
DA	0,22±0,04	0,32±0,17	0,32±0,05	0,46±0,19	0,602	0,214	0,653	0,551
HVA	0,13±0,08	0,05±0,40	0,05±0,01	0,06±0,02	0,399	0,383	0,860	0,787
3-MT	0,13±0,08	0,05±0,01	0,05±0,01	0,06±0,02	0,399	0,383	0,860	0,787
5-HIAA	3,08±0,29	2,53±0,16	3,15±0,18	2,51±0,11	0,155	0,876	0,896	**0,03**
5-HT	4,14±0,57	3,26±0,23	4,12±0,21	3,02±0,16	0,234	0,97	0,414	**0,004**

Note: designations as in Table 1.

Madopar increased DA and its metabolites concentration in all studied brain structures both in WAG/Rij and Wistar rats (Table 1 - Table 2). It should be noted, that the prefrontal cortex was more sensitive to madopar compared with other brain structures. Apart from increases of DA and its metabolites concentration, the compound also increased 5-HIAA and decreased 5-HT concentration which led to a leveling of the initial differences between WAG/Rij and Wistar rats (Table 1). Since NA is synthesized from DA by the enzyme of dopamine-β-hydroxylase, an increase of NA concentration together with increase of DA seems to be logically expected. The changes of 5-HT and 5-HIAA concentrations in the prefrontal cortex elicited by madopar administration are apparently related with interaction and interactive regulation of activity of DA and 5-HT modulatory systems.

The second series of experiments.

The study of initial differences between 2-month old control WAG/Rij and Wistar rats in the second series of experiments (Table 2) mostly coincided with the results obtained in the first series of experiments (Table 1).The changes in concentration of monoamines and their metabolites in WAG/Rij and Wistar rats were mostly expressed in the prefrontal cortex (Table 2). The significant differences were found in the concentrations of NA, DOPAC, HVA and 5-HIAA. The amount of DOPAC and HVA was lower in WAG/Rij rats compared to Wistar rats. The same differences were found in 2-month old WAG/Rij rats (Table 1), and in adult WAG/Rij rats with extensive absence epilepsy compared to non-epileptic Wistar rats [41]. These differences between WAG/Rij and Wistar rats we consider as a rapid reaction of the prefrontal cortex because there were no difference

between 2 months old WAG/Rij and Wistar rats in the nucleus accumbens, hypothalamus and hippocampus. Only in the striatum a low concentrations of HVA and 5-HIAA (Table 2) was detected.

On the second day, only the trace effect of madopar administration was revealed (Table 2). In the prefrontal cortex, the concentration of 5-HIAA was reduced in control WAG/Rij and Wistar rats relative to madopar-treated WAG/Rij and Wistar rats. In the nucleus accumbens, no differences were found. In the hypothalamus, on the second day DOPAC concentrations in the control rats were lower than in madopar-treated groups. The difference between the control and madopar-treated WAG/Rij rats was detected in the striatum by the concentration of 5-HIAA (Table 2). In the hypothalamus there were differences between madopar-treated WAG/Rij and Wistar rats on DOPAC concentration. In the hippocampus, the differences between the madopar-treated WAG/Rij and Wistar rats were found on concentrations of HA, 5-HIAA and 5-HT.

4. Discussion

In the open field test, behavioral measures indicating anxiety didn't differ significantly between 2 months old vehicle-treated WAG/Rij and Wistar rats. After madopar injection latency to leave the center of the open field increased in WAG/Rij rats, while in Wistar rats, motor activity (number of squares crossed) and rearing decreased. Increase of latency to leave the center of arena is considered to be an index of anxiety and fear [9,41] and reflects freezing reaction which appear in response to stress [30]. So, a low dose of madopar increased anxiety in WAG/Rij rats, and reduced motor and explorative activity (number of rearing) in Wistar rats, which may also reflect an increased level of anxiety. On the second day of testing, animals habituated to experimental situation. They became less anxious and expressed less fear as revealed by reduction the latency to leave the center of arena. The activating effects of madopar on the second day of testing were seen only in WAG/Rij rats: they left the center of an open field faster and showed more horizontal/vertical activities and grooming reactions relative to Wistar rats.

In the light–dark choice test of anxiety, 2-month old vehicle-treated WAG/Rij rats did not differ from the corresponding group of Wistar rats by 5 out of 6 behavioral parameters. However, one of the most appropriate (ethologicaly valid) index of anxiety in this test - the number of "risk assessments" [10,40,41] indicated an increased anxiety in vehicle-treated WAG/Rij rats compared to their Wistar counterparts. Madopar increased anxiety in both strains of rats, with more expressed effects in WAG/Rij rats, which spent less time in the light compartment of the chamber; and showed less number of transitions between compartments and rearing in the light compartment. The number of "risk assessments" in WAG/Rij rats was higher compared with Wistar rats both after vehicle and madopar injections.

In the forced swimming test, no significant differences in the immobility duration were found between 2-month vehicle-treated WAG/Rij and Wistar rats. However, in the vehicle-treated WAG/Rij rats, the first episode of active swimming was significantly shorter than in Wistar rats. The first episode of active swimming ('climbing', 'struggling') is a primary response of animals to stress caused by forced swimming (see [37,41]. This behavioral index in this test is particularly sensitive to stress. Therefore, the shorter duration of the first episode of active swimming in WAG/Rij rats may be related to their higher reactivity to stress and increased anxiety (passive coping strategy) in comparison to Wistar rats. Madopar produced an antidepressant-like effect (decreased immobility duration and number of head twitching), but only in WAG/Rij rats. In favor of antidepressant-like effect of madopar in WAG/Rij rats also indicates a strengthening of active swimming movements ("climbing", 'struggling', jumping), although the duration of the first episode of active swimming was unchanged. Madopar reduced the number of boli and head twitching in WAG/Rij rats. We assume that the number of head twitching is an index of stress-reactivity caused by forced swimming, though some authors consider it as an index of depression: the lower the score of head twitches, the lower a level of depression and vice versa [29].

Thus, the general conclusion deriving from the data obtained is that in 2-month old WAG/Rij rats we observe the very beginning stage of the pathology development which is characterized by increased anxiety and stress–reactivity. At age of 36 days, when phenotypic expression of absence epilepsy in WAG/Rij rats is absent, neurochemical alterations in the brain suggesting a hypo-function of the mesolimbic dopaminergic system (deficit of dopamine in the nucleus accumbens), as well as symptoms of depression-like behavior, are not detected [43]. At age of 5-6 months, WAG/Rij rats express strongly pronounced depression-like behavior [41].

2-month old WAG/Rij and Wistar rats after both vehicle- and madopar administration did not display any difference in passive avoidance learning. At the age of 5-6 months, WAG/Rij rats showed memory deficit in this task compared to their Wistar counterparts. This deficit was alleviated by pharmacological treatment which induced increases in DA concentration in the brain structures [7].

In active avoidance conditioning, 2-month old WAG/Rij rats showed better learning on the first day, and memory deficits on second day compared to Wistar rats. Increases in DA concentrations in the brain structures induced by madopar, in WAG/Rij rats leads to a strengthening the learning parameters on the first day and to a some weakening of memory deficits on the second day. Madopar administration does not affect the processes of learning and memory in 2-month old Wistar rats. In adult 5-6 month old WAG/Rij rats [7], modifications of active avoidance learning and memory processes were similar to those observed in 2-month old WAG/Rij rats, but in younger animals processes were somewhat different.

On the basis of learning and memory experiments, one can conclude that 2-month old WAG/Rij rats start to acquire a pathological state related with cognitive impairment, since at this age no difference between them and Wistar rats on passive avoidance performance was observed yet. The active avoidance conditioning was more sensitive test to detect violations of learning and memory in WAG/Rij rats compared with passive avoidance conditioning.

The results of neurochemical studies suggest that 2-month old WAG/Rij rats are characterized by monoamine

and their metabolites changes in the prefrontal cortex only. In other investigated brain structures differences between 2-month old WAG/Rij and Wistar rats were not revealed, with the exception of increases in HVA and 5-HIAA concentrations in the striatum. In adult 6-month old WAG/Rij rats, the neurochemical changes compared with Wistar rats occurred in all investigated brain structures [42]. Moreover, the deficits of the mesolimbic DAergic brain system showed age-related aggravation which was associated with increases in depression-like symptoms [43]. In other words, the beginning of the pathological process in 2 months old WAG/Rij rats related with deficiency of DA in the prefrontal cortex, is manifested at the behavioral level in the form of increased anxiety/stress reactivity and learning and memory impairment in the passive avoidance test. However, with time, during the development of pathologic process, dysfunction of DA-ergic brain system increases and leads to a reduction of anxiety and stress-reactivity typical for 2-month old WAG/Rij rats on the one hand, and to the development of pronounced depression-like behavior and increases in learning and memory deficits on the other hand [7,41,42,43]. The data that at age of 6 months when the disease process has reached its maximal development in WAG/Rij rats signs of increased anxiety and stress-reactivity are not present both at the behavioral and hormonal (corticosterone) levels [41] can be served as a confirmation of this assumption.

Taken together, results suggest that two month-old WAG/Rij rats are at the stage of so called “pre-pathology” (increased anxiety and stress reactivity) preceding the development of depression-like behavior and substantial cognitive impairments which are co-morbid to fully expressed absence epilepsy in 5-6 months old rats of this strain.

A rapid reaction of the prefrontal cortex to madopar administration related with increase of DA, its metabolites and NA, and decrease of 5-HT. The rapid reaction of the prefrontal cortex was also seen in irradiation experiments with radioactive carbon ions [^{12}C] on metabolism of monoamines in rat’s brain [Matveeva et al., 2013]. Although the experimental conditions were different, the neurochemical and behavioral changes were very similar. A few months after exposure to heavy particles [^{12}C] severe impairments of neurotransmitters concentrations, behavioral characteristics and learning and memory deficits were observed (for details see [26]). As in the case of WAG/Rij rats these disturbances were strengthen by time. The neurochemical changes appeared a day after irradiation by decrease of monoamines and their metabolites in the prefrontal cortex only [26].

The prefrontal cortex, ventral striatum or nucleus accumbens, hypothalamus, hippocampus and dorsal striatum are the fundamental structures for realization of voluntary and purposeful (emotional and motivational) behavior. The principle role in interaction of these structures plays NA, DA and 5-HTsystem [2,3,4,5,6]. Dorsal striatum executes a motor control, mediates voluntary behavior, receiving glutamatergic synaptic activation from cortex; integrates it and triggers thalamocortical network through the globus pallidus, and transfers processed and integrated information to motor cortex, which directly implements behavior [6]. The process of information integration in the dorsal striatum is under control of prefrontal cortex and the compact part of the substantia nigra (Nigro-striatal DA-ergic system) [6,27].

The nucleus accumbens or ventral striatum is the center of integration for mesolimbic DA-ergic system [11]. This nucleus integrates information from the hypothalamus, amygdala, hippocampus,habenula and prefrontal cortex [22,24]. In the hypothalamus are localized the centers of eating, drinking and sexual behavior [1,28,33]. Activated by aversive stimuliamygdala [22] and habenula [24,44] through interaction with the ventral tegmental area and ventral striatum, provide reinforcement at defensive and aversive reactions. Violation of integration processes in the meso-limbic system causes pathological states including depression [11,24]. The hippocampus is responsible for processing of spatial information (spatial memory) and contextual cues [11].

In our experiments, a rapid reaction of the prefrontal cortex relative to other brain structures was found (Table 1, Table 2), as well as in the study of Matveeva et al., [26]. It was shown that rapid reactions are a fundamental property of the prefrontal cortex. An increased reactivity of the prefrontal cortex to stress in rats was shown in several studies, for instance in Kings et al. [23]. The medial prefrontal cortex of rats is one of the most important system components of a quick learning which retrieves recent and old memories [17].

The cellular mechanisms of the prefrontal cortex neurons contributing to implementation of the rapid reactions are also found. Interactions between the rapid spike inhibitory interneurons and excitatory pyramidal neurons in rats facilitate implementation of fundamental properties of cortical networks. A key role of rapid spike interneurons is to provide a rapid inhibition in local networks of sensory and motor cortex and processing of input information from the thalamus to cortex [47]. Arapid DA-ergic modulation of calcium and rapid potentials in dendrites of the prefrontal cortex pyramidal in rats was revealed [48]. In the prefrontal cortex DA-ergic modulation occurs in less than 0.5 seconds, whereas in other structures it takes several seconds.

Apart from the fact that the prefrontal cortex forms and controls emotional and motivational states [19,21], it plays a key role in cognitive processes [21,45]. If cognitive processes in human related with acquisition of knowledge and experience for further application, in animals it is mainly gainedfor experience and future use. Another major function of the prefrontal cortex in implementation of animal’s behavior is a decision making and action selection [8,21], also associated with cognition [21]. We assume that the rapid reaction of the prefrontal cortex acquires a particular importance duringdecision making and actions selection. To survive, an animal needs to make a fast and accurate decision under uncertainty conditions in a permanently changing world [14].

5. Conclusions

Thus, on the basis of the data obtained we can conclude that at the age of 2 months WAG/Rij rats get into the initial stage of pathology (the so-called stage of pre-pathology), because their behavior acquires signs of increased anxiety and stress reactivity (but not depression),

no changes in passive avoidance and violation of active avoidance conditioning. The neurochemical changes occurred exclusively in the prefrontal cortex, which called as a rapid reaction of the prefrontal cortex. The pre-pathology stage in WAG/Rij rats probably is shaped by the rapid reaction of the prefrontal cortex. This fundamental property of the prefrontal cortex remains at the late stages of absence epilepsy, probably due to an impairment of the DA-ergic meso-cortico-limbic system, increase of depression-like behavior and disrupting of learning and memory in 5-6-monthold WAG/Rij rats. A single administration of L-DOPA precursor of DA synthesis (madopar) increased concentration of DA in the meso-cortico-limbic and nigro-striatal DA-ergic brain systems and modified behavior of WAG/Rij and Wistar rats. The WAG/Rij rats were more sensitive to madopar administration than Wistar rats. On the second day after madopar administration only the traces of its effects have been retained.

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References

[1] Barson JR, Morganstern I, Leibowitz SF (2011). Similarities in hypothalamic and mesocorticolimbic circuits regulating the overconsumption of food and alcohol. Physiol Behav; 104 (1): 128-137.

[2] Bazyan AS (1999). Neuromodulators and integrative mechanisms of emotional and motivation states. Neurochemical Journal; 16 (2): 88-103.

[3] Bazyan AS, Orlova NV, Getsova VM (2000). Modulation of the activity of monoaminergic brain systems and emotional condition by dalargin in rats during development of emotional resonance response. Zh Vyssh Nerv Deiat Im I P Pavlova; 50(3): 500-508.

[4] Bazyan AS (2001). Divergent and convergent mechanisms of the integrative activity of the mammalian brain. Zh Vyssh Nerv Deiat Im I P Pavlova; 51(4): 514-528.

[5] Bazyan AS, Segal OL (2009). Synaptic and paracrine nonsynaptic systems of the mammalian brain. Neurochemical Journal; 3(2): 77-86.

[6] Bazyan AS, Grigir'ian GA, Ioffe ME, 2011. Regulation of motor behaviour. Usp Fiziol Nauk; 42(3): 65-80.

[7] Bazyan AS, and van Luijtelaar G (2013). Review article. Neurochemical and behavioral features in genetic absence epilepsy and in acutely induced absence seizures. ISRN Neurology. Art. ID 875834.

[8] Bechara A, Damasio AR (2005). The somatic marker hypothesis: a neural theory of economic decision. Games and Econom. Behav; 52 (2): 336-372.

[9] Braw Y, Malkesman O, Dagan M, Bercovich A, Lavi-Avnon Y, Schroeder M, Overstreet DH, Weller A (2006). Anxiety-like behaviors in pre-pubertal rats of the Flinders Sensitive Line (FSL) and Wistar-Kyoto (WKY) animal models of depression. Behav. Brain Res; 167 (2): 261-269.

[10] Calatayud F, Belzung C, Aubert A (2004). Ethological validation and the assessment of anxiety-like behaviors: methodological comparison of classical analysis and structural approaches. Behav Processes; 67(2): 195-206.

[11] Carlezon WAJr, Thomas MJ (2009). Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis. Neuropharmacol; 56 (1): 122-132.

[12] Costall B, Jones BJ, Kelly ME, Neylor RJ, Tomkins DM, (1989). Exploration of mice in a black and white test box: validation as a model of anxiety. Pharmacol Biochem Behav; 32(3): 777-785.

[13] Cryan JF, Lucki I (2000). Antidepressant-like behavioral effects mediated by 5-hydroxytryptamine (2C) receptors. Pharmacol. Exp. Ther; 295(3): 1120-1126.

[14] Deneve S, (2012). Making decisions with unknown sensory reliability. Front Neurosci. 6:75.

[15] Espejo EF (1997). Selective dopamine depletion within the medial prefrontal cortex induces anxiogenic-like effects in rats placed on the elevated plus maze. Brain Res; 762(1-2): 281-284.

[16] Garcia-Cairasco N, Oliveira JAC, Wakamatsu H, Bueno ST, Guimaraes FS (1998). Reduced exploratory activity of audiogenic seizure susceptible Wistar rats. Physiol Behav; 64(5): 671-674.

[17] Gonzalez C, Kramar C, Garagoli F, Rossato JI, Weisstaub N, Cammarota M, Medina JH **(**2013). Medial prefrontal cortex is a crucial node of a rapid learning system that retrieves recent and remote memories. Neurobiol. Learn. Mem; 103: 19-25.

[18] Griebel G., Rodgers R., Perrault C.H, Sanger D.J., 1997. Risk assessment behavior: evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze test. Pharmacol Biochem Behav. 57(4): 817-827.

[19] Gruber A.J., McDonald R.J. 2012. Context, emotion, and the strategic pursuit of goals: interactions among multiple brain systems controlling motivated behavior. Front Behav Neurosci; 6: 50.

[20] Hascoët M., Bourin M., Nic Dhonnchadha B.Á., 2001. The mouse ligth-dark paradigm: A review. Prog. Neuropsychopharmacol. Bio. Psychiatry; 25(1): 141-166.

[21] Ho S.S., Gonzalez R.D., Abelson J.L., Liberzon I., 2012. Neurocircuits underlying cognition-emotion interaction in a social decision making context. Neuroimage; 63(2): 843-537.

[22] Jüngling K, Seidenbecher T, Sosulina L, Lesting J, Sangha S, Clark SD, Okamura N, Duangdao DM, Xu Y-L, Reinscheid RK, Pape H-C (2008). Neuropeptide S-mediated control of fear expression and extinction: Role of intercalated GABAergic neurons in the amygdala. Neuron; 59(2): 298–310.

[23] King D, Zigmond MJ, Finlay JM (1997). Effects of dopamine depletion in the medial prefrontal cortex on the stress-induced increases in extracellular dopamine in the nucleus accumbens and shell. Neurology; 77(1): 141-153.

[24] Li B, Piriz J, Mirrione M, Chung C, Proulx CD, Schulz D, Henn F, Malinow R (2011). Synaptic potentiation onto habenula neurons in learned helplessness model of depression. Nature; 470(7335): 535-539.

[25] Lucki I (1997). The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav. Pharmacol; 8(6-7): 523-532.

[26] Matveeva MI, Shtemberg AS, Timoshenko GN, Krasavin EA, Narkevich VB, Klodt PM, Kudrin VS, Bazyan AS (2013). The effects of irradiation by ^{12}C carbon ions on monoamine exchange in several rat brain structures. Neurochemical Journal; 7(4): 303–307.

[27] Mink JW (2003). The basal ganglia. Fundamental neuroscience. 2nd/Ed. Scuire LR, Bloom FT, McConnell SC, Roberts JL, Spitzer NC, Zigmond MJ. Elsevier Sci.: Acad. Press; 815-839.

[28] Morrow A.L., Porcu P., Boyd K.N., Grant K.A., 2006. Hypothalamic-pituitary-adrenal axis modulation of GABAergic neuroactive steroids influences ethanol sensitivity and drinking behavior. Dialogues Clin. Neurosci; 8(4): 463-477.

[29] Naitoh H, Nomura S, Kunimi Y, Yamaoka K (1992). "Swimming-induced head twitching" in rats in the forced swimming test induced by overcrowding stress: a new marker in the animal model of depression? Keio J Med; 41(4): 221-224.

[30] Nozek K, Dennis K, Andrus BM, Ahmadiyeh N, Baum AE, Woods LC, Redei EE (2008). Context and stress-dependent behavioral response to stress. Behav. Brain Functions; 4:23.

[31] Porsolt RD, Lenegre A (1992). Behavioral models of depression. In Elliot J.M., Heal D.J., Marsden C.A. (Eds) Experimental approaches to anxiety and depression. Willey, New York; 73-85.

[32] Prut L, Belzung C (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol; 463(1-3): 3-33.

[33] Przybycien-Szymanska MM, Gillespie RA, Pak TR (2012). 17β-Estradiol is required for the sexually dimorphic effects of repeated binge-pattern alcohol exposure on the HPA axis during adolescence. PLoS One; 7 (2):e32263.

[34] Sarkisova KYu, Kolomeitseva IA, Kulikov MA (1996). Effects of substance P on behavioral measures in the open field and forced swimming tests in rats with different type of behavior. Bull Exp Biol Med; 121: 244-247.

[35] Sarkisova KYu, Kulikov MA, (1994). Individual differences in reaction to acute stress associated with type of behavior. Prediction of the resistance to stress. Bull Exp Biol Med; 117: 89-92.

[36] Sarkisova KYu, Kulikov MA (2001). Prophylactic action of the antioxidant agent AEKOL on behavioral (psycho-emotional) disturbances induced by chronic stress in rats. Neurosci Behav Physiol; 31(5): 503-508.

[37] Sarkisova KYu, Midzyanovskaya IS, Kulikov MA (2003). Depressive-like behavioral alterations and c-fos expression in the dopaminergic brain regions in WAG/Rij rats with genetic absence epilepsy. Behav Brain Res; 144(1-2): 211-226.

[38] Sarkisova KYu, Kulikov MA (2006). Behavioral characteristics of WAG/Rij rats susceptible and non-susceptible to audiogenisc seizures. Behav Brain Res; 166(1): 9-18.

[39] Sarkisova KYu, Kulikov MA, Midzyanovskaya IS, Folomkina AA (2008). Dopamine-dependent nature of depression-like behavior in WAG/Rij rats with genetic absence epilepsy. Neurosci Behav Physiol; 38(2): 119-128.

[40] Sarkisova KYu, Kulikov MA, Folomkina AA (2011). Does antiabsence drug ethosuximide exert antidepressant effect? Zh Vyssh Nerv Deiat Im I P Pavlova: 61(2):227-235.

[41] Sarkisova K, van Luijtelaar G (2011). Review article. The WAG/Rij strain: a genetic animal model of absence epilepsy with comorbidity of depression. Prog. Neuro-Psychopharmacol. Biol. Psychiatry; 5(4): 854-876.

[42] Sarkisova KYu, Kulikov MA, Kudrin VS, Narkevich VB, Midzyanovskaya I.S., Birioukova LM, Folomkina AA, Bazyan A.S (2013a). Neurochemical mechanisms of depression-like behavior in WAG/Rij rats. Zh. Vyssh. Nerv. Deiat. Im I P Pavlova; 63(3): 303-315.

[43] Sarkisova KYu, Kulikov MA, Midzyanovskaya IS, Birioukova LM, Kudrin VS (2013b). Neurochemical mechanisms of depression in absence epilepsy. Abstracts IX International interdisciplinary congress "Neuroscience for medicine and psychology", Sudak, Crimea, Ukraine, June 3-13, P. 279-280.

[44] Shumake J, Ilango A, Scheich H, Wetzel W, Ohl FW (2010). Differential neuromodulation of acquisition and retrieval of avoidance learning by the lateral habenula and ventral tegmental area. J Neurosci; 30(17): 5876-5883.

[45] Tanji J, Hoshi E (2008). Role of the lateral prefrontal cortex in executive behavioral control. Physiol Rev; 88(1): 37-57.

[46] Takao K., Miyakawa T., 2006. Light/dark transition test for mice. J Vis Exp. Nov; 13(1): 104.

[47] Zaitsev AV, Lewis DA (2013). Functional properties and short-term dynamics of unidirectional and reciprocal synaptic connections between layer 2/3 pyramidal cells and fast-spiking interneurons in juvenile rat prefrontal cortex. Eur J Neurosci; 38(7): 2988-2998.

[48] Zhou WL, Antic SD (2012). Rapid dopaminergic and GABAergic modulation of calcium and voltage transients in dendrites of prefrontal cortex pyramidal neurons. J Physiol; 590(16): 3891-3911.