PHYSIOLOGY

Influence of subchronic exposure to manganese carried by female rats during pregnancy on the behavioral and cognitive abilities of their offspring at later stages of postnatal development

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Abstract

The study evaluated the effects of prenatal exposure to manganese (Mn) on the functional state of offspring at later stages of postnatal development (PND). Female rats were treated with $MnSO_4 \cdot 5H_2O$ in the diet at a dose of 1433 mg/kg starting 28 days prior to breeding and through gestation. The pregnancy proceeded normally, no physical abnormalities were observed. There was a lag in physical development of the offspring, which was characterized by a later opening of the eyes, the formation of a coat and the eruption of incisors, the weight gain was attenuated from PND 0–84. Offspring had hyperactive behavior and deterioration in spatial learning and memory. The level of Mn in the blood serum and cerebral cortex was higher than the control values by 11 and 53 % respectively. The acetylcholinesterase level in the serum was higher by 47 %. These findings highlight the risk of prenatal exposure to subchronic doses of Mn.

Keywords: heavy metals, manganese, toxicity, prenatal influence, behavior, cognitive abilities, acetylcholinesterase, biomarker

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Introduction

It is known that manganese (Mn) is an essential trace element necessary for the normal functioning of various physiological processes, including the metabolism of amino acids, lipids, proteins and carbohydrates. However, along with this, excessive exogenous exposure to this chemical element can lead to the development of systemic disorders in the human and animal organisms (Radysh et al., 2017; Oberlis, Harland, and Skalny, 2018).

It is interesting to note that, along with occupational exposure to Mn, there is an increased risk of chronic exposure to relatively low concentrations of this metal in the general population. This is confirmed by the data of environmental monitoring, during which it was established that the maximum permissible concentrations of Mn in the atmospheric air, soils, as well as in drinking and domestic water in many regions of the Russian Federation (RF) were exceeded (Chernogaeva et al., 2022). In addition, according to a comprehensive survey, an increased content of Mn in the hair of residents of various sex and age groups was registered in a number of federal districts of the Russian Federation (Skalny et al., 2011, 2012, 2013, 2014). In this regard, in recent decades, scientists have begun to pay special attention to the study of the consequences of chronic low-level exposure to Mn on the human and animal organisms (Korchina, Minyaylo, and Korchin, 2018; Fernández-Olmo et al., 2021; Racette et al., 2021).

It should be emphasized that exposure to Mn is especially dangerous in early ontogeny, i. e., during prenatal and early postnatal development. As is known, the

prenatal period plays a key role in the development of the organism. At this stage, the fetus is the most vulnerable and sensitive to the effects of any adverse factors, including excessive intake of Mn into the maternal organism. The results of numerous studies indicate that adverse environmental conditions and stress during fetal development can lead to a whole range of functional and structural disorders in body systems (Scott-Goodwin, Puerto, and Moreno, 2016; Gonzalez-Casanova et al., 2018). Thus, modern experimental and clinical studies have shown the development of pathological changes in the functional parameters of the brain during early postnatal ontogenesis in response to acute exposure to Mn in the prenatal period (Andiarena et al., 2020; Beasley et al., 2022). However, the effects of low-level Mn exposure during prenatal development on adaptive responses, behavior, and cognitive functions of the offspring are still far from being fully understood. There is an assumption that the consequences of subchronic exposure to Mn at the stages of critical development of fetal organs and functional systems may also come out in later periods of postnatal development of the organism. In particular, such exposure to heavy metal during pregnancy may become a possible risk factor for the occurrence of diseases of the central nervous system and lead to delayed subclinical neurological disorders in adult offspring. Understanding the mechanisms of such programming and the nature of interactions between specific and nonspecific processes that determine the disturbances that occur when exposed to Mn during critical periods of embryogenesis is one of the urgent tasks in modern biology and medicine.

In connection with the above, the aim of the study was to study the long-term effects of prenatal exposure to Mn on the functional state of offspring.

Materials and methods

Object of study. Experimental studies were carried out in the experimental biological clinic of the Federal Research Centre for Biological Systems and Agricultural Technologies of the Russian Academy of Sciences (Orenburg) on Wistar rats. Laboratory animals were obtained from the NPP "Nursery for Laboratory Animals" (Moscow region, Pushchino), which is a unique nursery for Russia and the Commonwealth of Independent States (CIS) for the industrial breeding and maintenance of laboratory rodents of the specific pathogen free (SPF) category.

Throughout the experiment, the animals were kept at a temperature of 22 ± 1 °C in plastic cages with sawdust bedding under artificial lighting (12-hour daylight hours) and forced-air ventilation. The animals were fed with a basic diet (BD), containing manganese in dose 0.0637 g/kg. The animals were given *ad libitum* access to drinking water and feeding.

Chemicals

Manganese sulfate (II) ($MnSO_4 \cdot 5H_2O$) was produced by JSC Vekton (St. Petersburg) with a certified purity of at least 98 %. Salt is a pale pink crystalline powder, readily soluble in water. The selection of the dose of introduction of salt into the diet of animals was carried out on the basis of data from the information systems GESTIS Substance Database and the European Chemicals Agency (ECHA), and also in accordance with the developed patent (patent no. 2794816).

Experimental design

Wistar female rats at the age of 12 weeks (generation F_0) were divided into control (n = 10) and experimental (n = 10) groups. Animals of the experimental group within 28 days (pregestational period) received the basic diet with the addition of manganese sulfate ($MnSO_4 \cdot 5H_2O$) at a dose of 1433 mg/kg. Animals of the control group were on the BD. Further, in order to obtain offspring, intact males were placed with females. Fixation of pregnancy was carried out by the method of vaginal smears (Vladimirskaya et al., 2011). The first day of pregnancy was determined based on the detection of spermatozoa in the smear of females. Presumed pregnant female rats continued to be exposed to nominal doses of $MnSO_4 \times 5H_2O$ during the entire gestation period (≈ day 23). During the pregestational and gestational periods, food intake, body weight were measured in females; the state of the reproductive system, the course and outcomes of pregnancy were analyzed.

Males were selected from the resulting offspring (F_1) and distributed into the appropriate groups — control (n = 10) and experiment (n = 10). One male offspring was randomly selected from each litter to form the groups used for the experiments. Postnatal day (PND) 0 was designated as the first day when the delivery of offspring was observed by 8:30 a. m. Before reaching sexually mature age, the rat pups were assessed by the morphofunctional state, including the study of the physical development of rat pups during breastfeeding (PND 0–21), weekly determination of body weight (PND 0–84). Upon reaching the 84-day age of postnatal development (PND 84), the behavior and cognitive abilities of the animals were assessed using specialized test systems (the Open Field test, the Morris Water Maze test). The animals were taken out of the experiment on PND 126. Blood was taken from the cardiac artery and samples of the cerebral cortex to determine chemical elements in these samples using ICP-DRC-MS, acetylcholinesterase (AChE) using enzyme immunoassay (EIA). Female rats were not involved into research due to the variable nature of female data caused by hormonal fluctuations associated with the female's reproductive cycle.

Behavioral and cognitive testing

The behavioral testing began when experimental animals reached PND 84 with at least one-week break period between consecutive assays for any individual rats.

The Open Field test is a method that is widely used in the study of behavioral responses in pharmacology, psychogenetics and animal psychology. It allows assessing neurotropic environmental factors (Gould, Dao, and Kovacsics, 2009; Kraeuter, Guest, and Sarnyai, 2019). The open field was a circular arena 100 cm in diameter with 13 holes in the floor and walls 30 cm high. All animals were placed one by one in the center of the test site, behavioral activity was registered for 3 minutes using a Logitech C920 PRO HD video system (China) for later analysis. After each test, the chambers were cleaned with a damp towel and 70 % alcohol. Data processing was carried out using the ToxTrac software (Rodriguez et al., 2018). The following indicators were assessed: horizontal locomotor activity, vertical locomotor activity, number of examinations of holes, grooming, vegetative activity, distance traveled, and movement speed.

The Morris Water Maze test is a well-established technique for assessing rodents' spatial learning and memory abilities (Othman, Hassan, and Che Has, 2022). The installation was a round plastic pool with a bottom diameter of 1.5 m and a side height of 0.6 m. The rat platform was made of Plexiglas. The water temperature was 24–28 °C; this level was constantly maintained by the water thermostat system. The pool was divided into four quadrants; they were arbitrarily projected northeast, northwest, southeast, and southwest.

In our study, a five-day test protocol was chosen. It included two stages: learning/training (from 1 to 4 days) and a trial test (5 days). The received video files were processed using ToxTrac software for tracking the behavior of animals (Rodriguez et al., 2018). The main parameters evaluated in the Morris water maze were the following: the distance traveled (cm) and the escape latency (sec).

Manganese analysis

Blood and brain sampling. Blood samples were taken from each rat from the cardiac artery in a vacuum tube, followed by separation of blood serum. Vacuette tubes with a blood clotting activator and a gel for separating red blood cell mass (Greiner Bio-One International AG, Austria) were used for blood sampling. After clotting the obtained blood samples were centrifuged at 1600 g for 10 min. The separated serum was stored at –70 °C in Eppendorf tubes until analysis. The samples were thawed at 4 °C overnight before analysis.

Cerebral cortex tissue was taken immediately after decapitation. The tissue section was made from the surface of the hemisphere with a thickness of about 1–1.5 mm, which approximately corresponded to the thickness of the cortex. The brain samples were weighed, and stored at –20 °C prior to ICP-DRC-MS analysis.

Serum and brain sample preparation. Serum samples were diluted 1 : 15 with an acidified (pH = 2.0) diluent containing (v/v) 1-Butanol 1 % (Merck KGaA, Darmstadt, Germany), Triton X-100 0.1 % (Sigma-Aldrich, Co., St. Louis, MO USA), and HNO3 0.07 % (Sigma-Aldrich, Co., St. Louis, MO, USA) in distilled deionized water.

Brain samples were rinsed with ice-cold distilled deionized water. All studied samples were added into Teflon tubes with concentrated HNO_3 for subsequent microwave digestion in Berghof speed wave (Berghof, Eningen, Germany) system for 20 min at 180 °C. After cooling the system, the obtained solutions were used for chemical analysis.

ICP-MS analysis. The analysis of manganese (Mn) levels in the studied samples was performed using inductively coupled plasma mass-spectrometry (ICP-MS) at NexION 300D (PerkinElmer Inc., Shelton, CT, USA) equipped with a 7-port FAST valve and ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA). External calibration of the ICP-MS system was performed with 0.5, 5, 10 and 50 μg/L solutions of the studied element. Calibration solutions were prepared from the Universal Data Acquisition Standards Kit (PerkinElmer Inc., Shelton, CT, USA). Internal on-line standardization was performed using 10 μg/L solutions of yttrium-89 and rhodium-103. The solutions were prepared from commercially available Yttrium (Y) and Rhodium (Rh) Pure Single-Element Standard (PerkinElmer Inc. Shelton, CT, USA) on a matrix containing 1-butanol 8 % (Merck KGaA, Gernsheim, Germany), Triton X-100 0.8 % (Sigma-Aldrich Co., St. Louis, MO, USA), tetramethylammonium hydroxide 0.02 % (Alfa Aesar, Ward Hill, MA, USA) and ethylenediaminetetraacetic acid 0.02 % (Sigma-Aldrich Co., St. Louis, MO, USA). The results of serum analysis were expressed as μg/mL for the element. Data on brain trace elements are provided for the element as μg/g.

Acetylcholinesterase analysis

Blood sampling Blood samples were taken from each rat from the cardiac artery in a vacuum tube, followed by separation of blood serum. Vacuette tubes with a blood clotting activator (Greiner Bio-One International AG, Austria) were used for blood sampling. After clotting the obtained blood samples were centrifuged at 1000g for 15 min at 2–8 °C. The supernatant was collected to carry out the assay.

Enzyme immunoassay Analysis. To determine the concentrations of rat serum AChE, the Rat AChE (Acetylcholinesterase) ELISA Kit (Elabscience, China) was used. The stages of EIA were carried out in accordance with the instructions.

Statistics

The obtained data were processed using the methods of variation statistics using the "STATISTICA 10" statistical package (StatSoft Inc., USA). The study results were stored and the material was preprocessed in the original Excel 2010 database (Microsoft, USA). The compliance of the obtained data with the normal distribution law was checked using the Shapiro-Wilk test of agreement. The hypothesis that the data belonged to a normal distribution was rejected in all cases with a probability of 95 %, which justified the use of non-parametric procedures for processing statistical populations (Mann-Whitney U-test). The data obtained are presented as median (Me) and 25^{th}–75^{th} centiles (Q_{25}–Q_{75}). In all procedures of statistical analysis, the achieved level of significance (p) was calculated, while the critical level of significance in this study was taken to be less than or equal to 0.05.

Results

Maternal parameters (F_0). All laboratory animals throughout the experiment showed no clear signal of pathology or abnormal behavior (aversion to food or water). The appearance of the animals did not differ from the control evaluating the functional indicators.

It was found that rats across the control and experimental groups exhibited different weight gain with no significant differences in volumes of food consumption. The weight of the experimental rats on 21 day (pre-breed period) and 35 day (pregnancy) of consumption the manganese-containing diet was with statistical significance lower than in the control group (Fig. 1A).

In female rats of the experimental group, against the background of subchronic oral exposure to Mn in the pregestational period, there was a lower fertility index and pregnancy index by 20 and 11.5 %, respectively.

When assessing the impact of Mn on the course and results of pregnancy, it was found that the pregnancy proceeded normally, the offspring showed no signs of physical abnormalities at birth, and no cases of stillbirth were recorded. However, the terms of delivery in the experiment were recorded earlier by 1.6 days (p = 0.04) relative to the control, however, it was within the normal range (Sharp and Regina, 1998); body weight of newborns and their size was significantly less than the control values by 20 % (p = 0.02) (Table).

Table. Evaluation of the impact of manganese on the course and results of pregnancy

Indicators	Groups	
	Control	Experimental
Pregnancy period, days	23.3 (22.9–23.9)	21.7 (20.8–22.2)*
Number of rat pups in a litter, pcs.	7.3 (6.7–8.5)	9.4 (8.2–10.3)
Body weight at birth, g	5.5 (4.6–6.3)	4.3 (3.6–4.9)*
Body size of rat pups at birth, cm	3.9 (3.7–4.2)	3.1 (2.8–3.5)*
Stillbirth, %	0	0

Note: The data obtained are presented as median (Me) and 25^{th}–75^{th} centiles (Q_{25}–Q_{75}); * — significant difference in comparison to the control at $p < 0.05$.

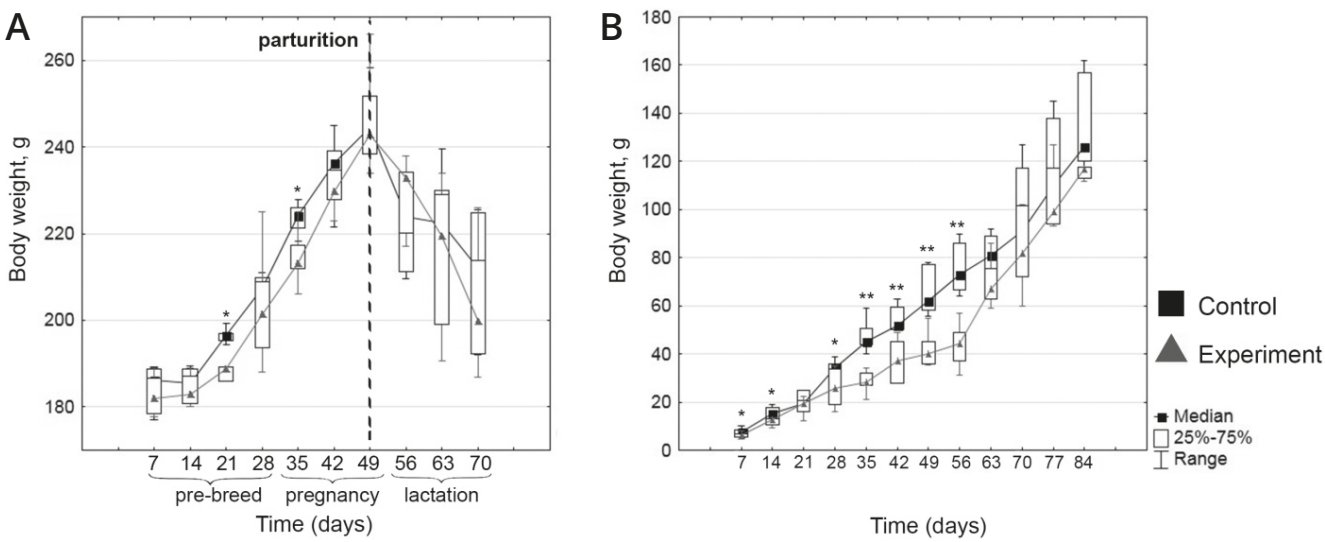

Fig. 1. A — body weights of female-rats exposed subchronic oral exposure to Mn in the pre-breed and gestational periods, g; B — body weights of offspring exposed maternally to Mn, g.

Note: The data obtained are presented as median (Me) and 25^{th}–75^{th} centiles (Q_{25}–Q_{75}); * — significant difference in comparison to the control at $p < 0.05$.

Offspring parameters (F_1)

Developmental parameters. Prenatal exposure to Mn led to a delay in the physical development of F1 animals, which was manifested by a later opening of the eyes (2.5 days later; $p = 0.03$), as well as a tendency to later appearance of the coat and eruption of the incisors compared with the control group. The body size of newborn animals of the experimental group was significantly less than the control by 20 % when comparing the medians (Table). Prenatal exposure to manganese affected the body weight of rat pups. At PND14, PND28, PND35, PND42, PND49, PND56 the weight of animals in the experimental group was significantly lower by 19, 24, 37, 29, 35 and 39 %, respectively, when comparing the median values (Fig. 1B).

Locomotor activity and anxiety in the open-field test. An increase in motor activity was registered in animals of the experimental group F_1. It was characterized by a significant increase in the distance traveled and an increase in the speed of movement of animals in the arena by 135 % ($p = 0.03$) and 55 % ($p = 0.01$), respectively, when comparing the median values. The number of fecal boluses increased — the median value was 100 % higher than the control values ($p = 0.01$) (Fig. 2).

Learning and memory in Morris Water Maze test. Figure 3A demonstrates the escape latency. The animals in the control group quickly mastered the test task and improved their skills during the training session. On the contrary, significant deviations from the control values were observed in the animals from the experimental group, starting from the first day of training. Experimental animals needed much more time to find a platform (Fig. 3A). However, according to the test results on the subsequent training (2nd, 3rd and 4th) days of the study, a decrease in the time of detection of the hidden platform was noted compared to the 1st day, which indicates that the rats retained the ability to learn and memorize.

On the fifth day, the animals were tested to reproduce the acquired skill. The animals of the control group successfully completed the task, purposefully moving to the sector and circulating in it, where the platform had previously remained, whereas the behavior of the experimental animals F_1 was less purposeful, the movement was chaotic. The animals swam into the sector where the platform was located during the training period, but,

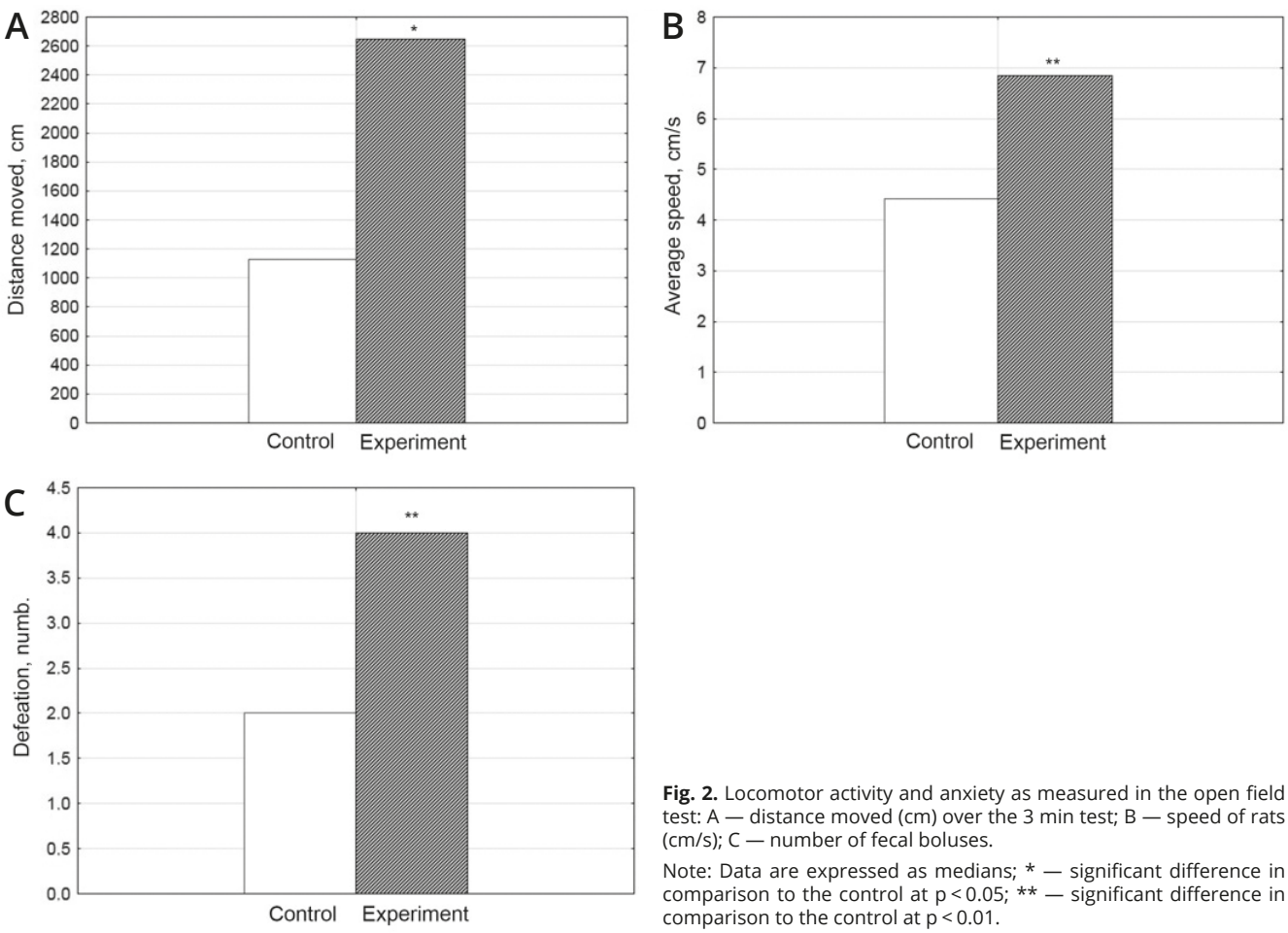

Fig. 2. Locomotor activity and anxiety as measured in the open field test: A — distance moved (cm) over the 3 min test; B — speed of rats (cm/s); C — number of fecal boluses.

Note: Data are expressed as medians; * — significant difference in comparison to the control at $p < 0.05$; ** — significant difference in comparison to the control at $p < 0.01$.

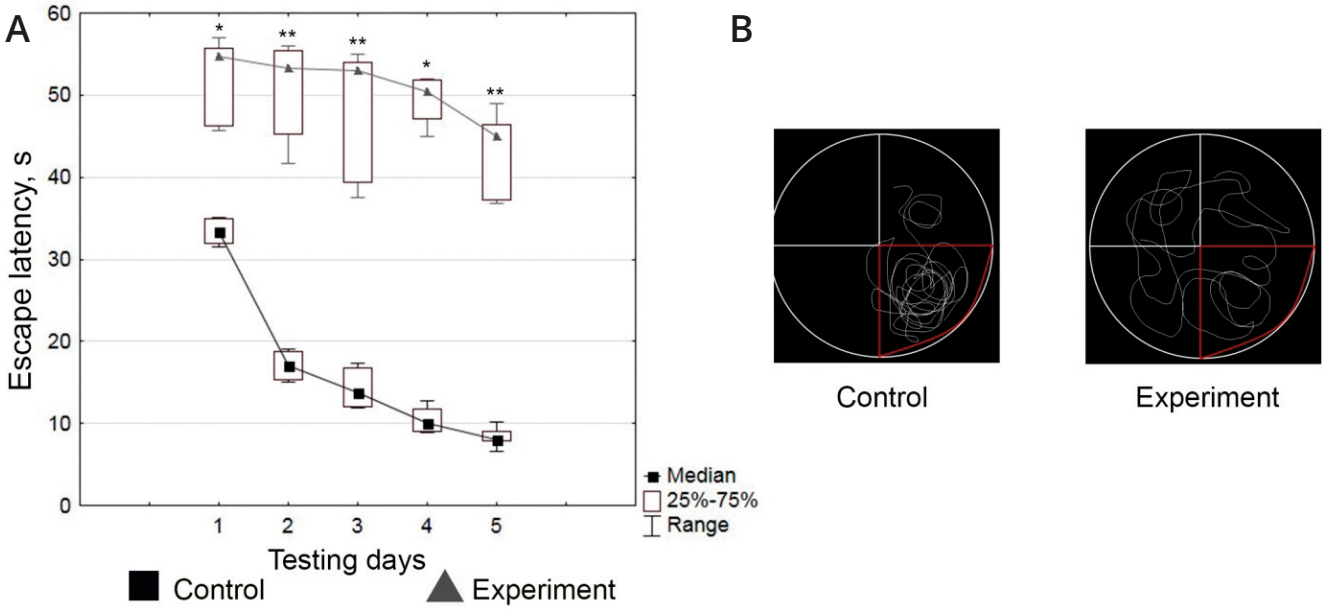

Fig. 3. Learning and memory as measured in Morris water maze test: A — escape latency of rats; B — representative swimming tracks of rats at Day 5.

Note: The data obtained are presented as median (Me) and 25th–75th centiles (Q_{25}–Q_{75}); * — significant difference in comparison to the control at $p < 0.05$; ** — significant difference in comparison to the control at $p < 0.01$.

unlike the control animals, did not linger in this area, passing it (Fig. 3B).

Thus, in animals of the first generation, a weakening of cognitive abilities was observed. It was accompanied by impaired spatial orientation and memory.

Manganese levels. The results of the study demonstrate that in adult offspring exposed to Mn during prenatal development, a higher concentration of this chemical element in the blood serum and cerebral cortex is observed in comparison with the control — higher by 11 % ($p = 0.05$) and 53 % ($p = 0.002$), respectively, when comparing the median values (Fig. 4A, B).

Acetylcholinesterase levels. According to enzyme immunoassay, the AChE level in the blood serum of adult offspring of the experimental group was significantly higher than the control values by 47 % ($p = 0.01$) when comparing the median values (Fig. 5).

Discussion

Since under natural conditions the exposure to Mn in the body is the result of a long process during which this heavy metal is able to accumulate, in the present study we used an experimental model with chronic exposure to Mn in laboratory rats. It was found that the weight of females of the experimental group F_0, exposed to chronic oral exposure to Mn, steadily increased throughout the experiment, including the pre-gestational period and the period of pregnancy. However, when compared with controls, a decrease in their body weight gain was recorded, indicating some toxic effects of the oral dose of Mn. Similar results were obtained in a study by Molina et al. (2011), during which the authors found a lower body weight of female rats exposed to Mn during pregnancy and lactation. In the course of experimental work, we also found that oral exposure to Mn in the pre- and gestational periods had negative consequences on the indicators of the generative function of animals, reducing the fertility and pregnancy index. The obtained results are consistent with the works of a number of authors, in which it was demonstrated that long-term exposure to Mn can lead to a decrease in the fertility of animals (Sengupt et al., 2015; Souza et al., 2019).

It is important to note that, despite the existence of numerous works in the field of studying the toxic effects of Mn on the body, there is still not enough data on the prenatal consequences of exposure to this metal using longitudinal research approaches. In this regard, one of the interesting areas of research in studying the vulnerability of a developing organism in animal experiments is the assessment of the long-term consequences of exposure to adverse environmental factors.

In the course of the experiment, it was found that animals that developed under conditions of prenatal exposure to Mn were inferior to the control ones in many respects. The weight and size of experimental animals at birth were significantly lower than in the control group by 20 %. There was a lag in physical development, which was characterized by a later opening of the eyes, the formation of a coat and the eruption of incisors. It should be noted that significant differences in weight persisted even in older animals (PND14, PND28, PND35, PND42,

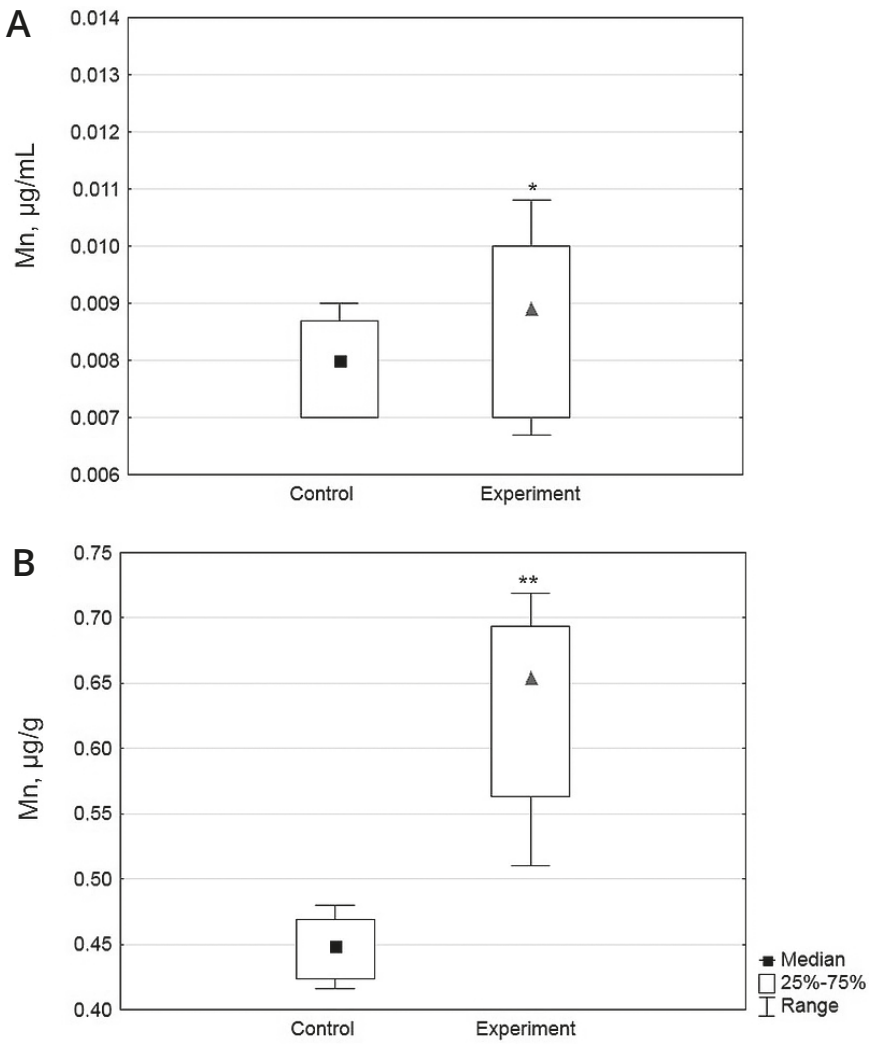

Fig. 4. Level of Mn in offspring: A — blood serum; B — cerebral cortex.

Note: The data obtained are presented as median (Me) and 25th–75th centiles (Q_{25}–Q_{75}); * — significant difference in comparison to the control at $p < 0.05$; ** — significant difference in comparison to the control at $p < 0.01$.

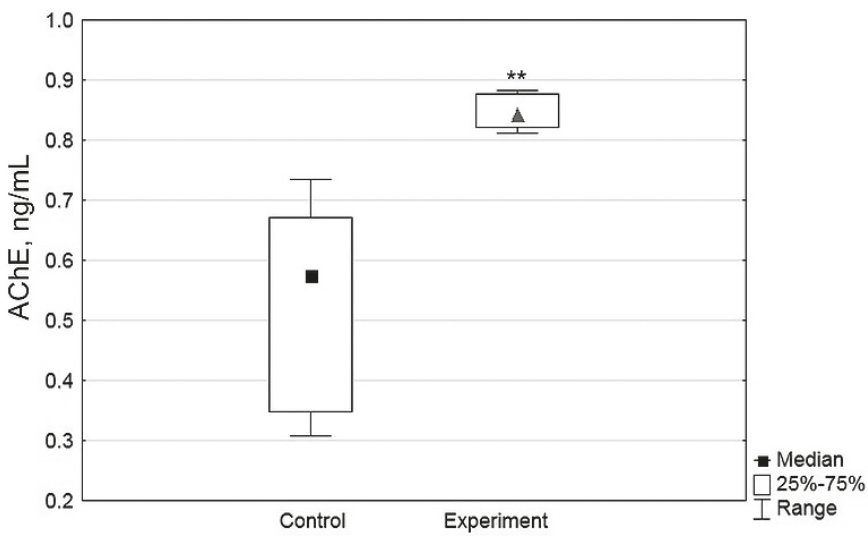

Fig. 5. Level of acetylcholinesterase in the blood serum of offspring.

Note: The data obtained are presented as median (Me) and 25th–75th centiles (Q_{25}–Q_{75}); ** — significant difference in comparison to the control at $p < 0.01$.

PND49, PND56). Analyzing the dynamics of the weight of rats during the entire experiment, a decrease in body weight gain was observed when compared with the control. The findings are consistent with the earlier works by Dorman et al. (2000) and Garcia, Gellein, Syversen, and Aschner (2006, 2007), as well as with more recent studies by Vorhees et al. (2014) and Amos-Kroohs et al. (2016), who reported weight loss in Mn-exposed offspring. One of the possible explanations for this effect may be oxidative stress that occurs against the background of excessive Mn intake, which results in impaired cellular functions and growth (Pizzino et al., 2017). However, along with this, there are opposite data. In the literature, there are works in which an increase in the weight of animals was found against the background of prenatal exposure to Mn, or there were no differences from control values at all (Tran et al., 2002; Molina et al., 2011). Such a discrepancy in the results obtained can be justified by the choice of different doses of the impact of this chemical element on the body. Thus, in clinical studies by a number of scientists, an inverted U-shaped relationship was found between the levels of Mn in the blood of matter during pregnancy and the body weight of infants (Ashley-Martin et al., 2018; Yamamoto et al., 2019). It has been shown that both deficient and surplus levels of Mn in maternal blood are associated with lower birth weight. We would like to emphasize that the comparison of studies on the effects of Mn is difficult due to the lack of consistency in the experimental methods. Nevertheless, at present, the problem of newborns with low and extremely low body weight is one of the key problems in neonatology and pediatrics. These children are at risk for the development of various chronic diseases (Kostina et al., 2017). Numerous scientific studies confirm that the functioning of the central nervous system of a child and indicators of childhood morbidity have a direct correlation with the degree of lag in metric parameters (Strizhakov, Ignatko, Timohina, and Kardanova, 2019). Low birth weight has been reported to be associated with adverse neurodevelopmental outcomes (Zavadenko, and Davydova, 2018).

The results of behavioral and cognitive studies have shown that exposure to Mn during prenatal ontogenesis leads to delayed deviations in behavioral responses, learning and memory disorders in animals.

The "Open field" test in this study was used to assess the locomotor activity and anxiety of animals (Gould, Dao, and Kovacsics, 2009). The test results showed that prenatal exposure to Mn contributes to the development of hyperactive behavior in rats at later stages of postnatal ontogenesis (PND 84), which may indicate the consequences of intoxication with this heavy metal in utero. A number of scientists have shown that Mn disrupts the balance of several neurotransmitter systems (dopamine and cholinergic), especially in the developing brain, and causes a wide range of behavioral disorders, including changes in sensorimotor function and cognitive abilities (Finkelstein, Milatovic, and Aschner, 2007; Santos et al., 2012; Yousefi Babadi et al., 2014; Schetinger et al., 2019). The data obtained are consistent with the studies of Chandra, Shukla, and Saxena (1979), Pappas et al. (1997), Brenneman, Cattley, Ali, and Dorman (1999) who found that prenatal and lactation exposure to Mn resulted in animals hyperactive at PND21, PND60, PND90. However, as noted by the scientists, at PND120 and later, no differences from the control in the behavior of animals were observed. At the same time, rather contradictory data are presented in the literature: the study by Dorman et al. (2000) noted no abnormalities in the motor activity of animals exposed to Mn, and in the work of Betharia and Maher (2012), on the contrary, hypoactivity and increased anxiety were observed on PND 24 and PND 59. Regardless of this, in general, the results of studies indicate a neurotoxic effect Mn.

The results of the Morris Water Maze test showed that prenatal exposure to Mn can have negative consequences on the state of the body's cognitive abilities at later stages of postnatal development, namely, lead to a deterioration in spatial learning and memory. The test results showed that adult animals developed under conditions of intrauterine exposure to Mn took longer to find the hidden platform, movement patterns were chaotic in the target quadrant when assessing spatial memory, indicating a violation of learning and memory processes. It is possible that such deviations arose due to the accumulation of this neurotropic metal element in the brain structures of animals associated with learning, memory and attention (cerebral cortex, basal ganglia, hippocampus), which, as a result, had an adverse effect on the formation of spatial skills (Su et al., 2016; Nyarko-Danquah et al., 2020). The results obtained are rather controversial and can form the basis for further research. In an experiment conducted by Pappas et al. (1997), in laboratory rats exposed to Mn during prenatal development and breastfeeding, on the contrary, there were no cognitive impairments at PND 25 and PND 95. However, it should be noted that the Mn exposure doses used in this study were lower than in our study. Along with it, in the work carried out by the American scientists, among the animals exposed to Mn during similar periods of development, memory impairment was revealed on PND25 and PND60 (Betharia and Maher, 2012). Similar results were established in the works of foreign scientists (Zhang, He, Zhang, and Tan, 1998; Zhang, He, Huang, and Li, 2001, 2002; Kern, Stanwood, and Smith, 2010). The inconsistency of the data obtained in both the Open Field test and the Morris Water Maze test may be due to the fact that the effects of Mn exposure largely depend on a number of concomitant factors, including the dose of exposure, duration of exposure, and the method of metal intake into the body, age and sex of experimental

animals, as well as individual sensitivity and metabolic detoxification capacity of the organism. It should be noted that in many studies, as a rule, the consequences of prenatal exposure to Mn in the early stages of postnatal development of animals were studied, without taking into account the later stages of ontogeny.

The results of the study also indicate that the deviations in the behavior of adult offspring whose mothers were exposed to Mn during pregnancy are obviously not due to a structural brain damage, but rather associated with neurochemical disorders caused by this metal. This statement is confirmed by the obtained results of elemental and enzyme immunoassay.

It was found that in the cerebral cortex of adult offspring exposed to prenatal exposure to Mn, the concentration of this metal significantly exceeded the control by 53 %. The established fact confirms the ability of Mn to penetrate the placenta and the blood-brain barrier (BBB) (Yoon et al., 2009). It is assumed that both the immaturity of the BBB and the increased need of the body for Mn during fetal development contribute to the entry of high concentrations of this chemical element into the brain of the developing fetus (Hu et al., 2018). The studies of Kontur and Fechter (1985), Pappas et al. (1997), as well as Molina et al. (2011) reported increased concentrations of Mn in the brain of newborn rats, as well as in the early stages of their postnatal ontogenesis, whose mothers consumed drinking water with a high content of Mn during pregnancy. The research of Garcia, Gellein, Syversen, and Aschner (2006, 2007) also recorded the accumulation of Mn in various brain structures of offspring whose mothers consumed a diet with a high content of Mn during pregnancy and lactation. However, it should be noted that in the conducted studies, as a rule, the content of Mn in the brain was determined at the early stages of postnatal development of the offspring (PND 21–32). Nevertheless, our results of the study show that the subtoxic Mn diet fed to female rats during the entire pregnancy leads to an increase in the Mn content in the cerebral cortex of adult offspring. It should be noted that, along with this, the level of serum Mn in the adult offspring of the experimental group was slightly higher than the control values (by 11 %), which is justified by the work of the hepatobiliary system, which is completely formed and regulates Mn concentrations during ontogenesis (Gurol, Aschner, Smith, and Mukhopadhyay, 2022).

Many scientific works are focused on the study of the effects of brain Mn accumulation on dopamine, glutamate and g-aminobutyric acid neurotransmission, because Mn neurotoxicity is linked with disturbances in motor and stereotypes behaviors. It is well known that Mn in the brain tissue affects neurotransmitter transport and protein functioning causing alterations in neurotransmitter levels (Lin et al., 2020; Spitznagel et al., 2023). However, data on changes in cholinergic systems of the central nervous system following the exposure to manganese are considerably less extensive (Koroleva, 2023). The high representation of cholinergic neurons in brain structures that play a key role in the formation of cognitive functions determines the critical importance of cholinergic transmission for memory, learning, and attention (Dziak, and Tsurkalenko, 2019). In this connection, we assessed the content in the blood serum of one of the most important enzymes of the cholinergic system — acetylcholinesterase. As is known, this enzyme (EC 3.1.1.7) belongs to the esterase family and participates in the termination of nerve impulse transmission due to the hydrolysis of the neurotransmitter acetylcholine in numerous cholinergic pathways in the central and peripheral nervous system (Starostina, and Degteva, 2008; Cheung et al., 2012; Notova, Karimov, Kazakova, and Marshinskaya, 2021). A number of works have established that AChE is sensitive to environmental factors and is a potential marker for assessing the impact of toxicants (Andrade et al., 2015; Fu et al., 2018; Dutta, and Bahadur, 2019). In some countries, the determination of the activity of this enzyme in the blood of patients exposed to adverse environmental factors (pesticides, heavy metals) is mandatory during a medical examination (Lionetto et al., 2013). It should be noted that Han et al. (2019) in their study showed that in addition to activity, it is also possible to use the assessment of the concentration of this enzyme as a potential biomarker to predict the development of disorders of the nervous system. In the studies of American scientists, the significance of the quantitative determination of AChE in amniotic fluid as a reliable diagnostic test for neural tube defects was also confirmed (Nørgaard-Pedersen, Hangaard, and Bjerrum, 1983; Rasmussen Loft et al., 1990). In the course of our analysis, it was found that the level of AChE in the adult offspring of the experimental group was statistically significantly higher than the control values by 47 %. Due to the fact that the levels of AChE enzymatic activity are assessed more often and the quantitative content of this enzyme is much less frequently determined, there is not enough information in the literature about the pool of AChE in various biosubstrates under pathological and normal physiological conditions (Ellman et al., 1961). A number of scientists have found inhibition of AChE activity after exposure to both acute and chronic doses of Mn in various biosubstrates (brain, blood, cerebrospinal fluid) of adult and newborn rats, however, there are no data on changes in the levels of the protein itself under such conditions in the available literature (Martinez, and Bonilla, 1981; Lai, Leung, and Lim, 1984; Okada et al., 2016). However, in the experimental work of Polish scientists, it was shown that in adult rats fed a diet with the addition of an increased content of Cu for two months, the concentration of AChE in the blood and brain in-

creased (Cendrowska-Pinkosz, Krauze, Juśkiewicz, and Ognik, 2021). As mentioned earlier, Mn exposure increases the risk of developing neurogenerative diseases (Bakulski et al., 2020; Spitznagel et al., 2023). It is interesting to note that in clinical and post-mortem studies in blood samples of patients with Alzheimer's disease and in the brain, despite a decrease in the enzymatic activity of AChE, elevated levels of this protein were found against the background of an increase in BBB permeability (Fishman et al., 1986; Kalaria, 1999; García-Ayllón et al., 2010; Kuvacheva et al., 2013; Campanari et al., 2014, 2016). The exact mechanism of the change in blood AChE levels remains unclear. However, in our study, it can be assumed that an increased level of Mn in the brain of rats acts as a chemical stressor for cholinergic neurons, initiating the formation of reactive free radical oxygen species and, as a result, leading to the development of an oxidative stress state, which is one of the triggers in functional impairment of organs and target systems (Erikson, Dobson, Dorman, and Aschner, 2004). This can disrupt the functioning of a number of synaptic proteins, including the AChE enzyme, as well as affect BBB permeability (Lehner et al., 2011). A decrease in AChE activity, in turn, contributes to excessive accumulation of the neurotransmitter acetylcholine in the nerve synapses of the peripheral and central nervous systems, hyperstimulation of nicotinic and muscarinic receptors, and, as a result, impaired nerve impulse transmission (Chtourou et al., 2012). It is known that the level of AChE synthesis and, accordingly, its amount is controlled by the pattern of synapse activity (Lømo, Massoulié, and Vigny, 1985). Therefore, increasing the frequency of their stimulation increases the synthesis of this AChE enzyme (Rotundo, 2003; Rotundo et al., 2008). Serum AChE is likely to have multiple cellular origins, including brain cells. Changes in the permeability of the BBB, both during physiological aging and during the development of pathological conditions of the CNS, can contribute to the movement of AChE through the BBB. The data obtained indicate that even minor changes in the work of the cholinergic system act as a "trigger" for the formation of neurological pathologies. It should be noted that disruption of the processes of barrier genesis during the development of the CNS can have a significant effect not only in the early postnatal period, but also in the long term of individual development. In one of the studies, it was shown that if this process is triggered in early ontogenesis, then it is able to persist throughout the adult life of the organism with a tendency to progress and globalize emerging disorders, even in the absence of an influencing factor (Bałasz et al., 2015).

Thus, the violations of behavior and ability to learn in adult animals exposed to Mn during prenatal development are accompanied by changes at the molecular and cellular level.

Conclusion

Thus, excessive exposure to Mn during pregnancy has an adverse effect on the basic molecular and cellular mechanisms of the offspring, which subsequently leads to significant changes in the functioning of the brain.It is reflected in the behavioral level and learning ability of animals at later stages of postnatal ontogenesis. Consequently, the postnatal period is the implementation of the prenatal program of the physical, somatic and mental health of the body. Based on this study, it can be concluded that prenatal exposure to Mn may pose a lifelong risk of neurological disorders. The progressive and latent nature of some higher nervous disorders suggests that the triggering event for such disorders occurs much earlier than the onset of visible symptoms. Therefore, it is important to identify possible environmental triggers, as well as predictors of such disorders, which will help reduce not only perinatal mortality, but also many postnatal diseases, including those that manifest only in middle and adulthood.

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