THE INFLUENCE OF CHRONIC PRENATAL HYPOXIA ON THE FUNCTIONAL STATE OF MICE AND THEIR ADAPTATION TO AUDIOGENIC SEIZURES

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Abstract. Prenatal hypoxia remains the leading cause of infant mortality and severe disability in newborns. Disturbances in the development of fetal brain structures and functions due to hypoxic damage are the main trigger for the development of severe neurological disorders and accelerated neurodegeneration processes and can also be the cause of epileptiform activity in the postnatal period. Herein, the role of chronic prenatal hypoxia on the functional state of C3H+C57Bl6 hybrid mice during the first three weeks of postnatal development and the risks of developing epileptiform activity when provoking audiogenic seizures were assessed. Exposure to chronic prenatal hypoxia was found to increase the risk of neonatal mortality and developmental delay in the surviving individuals in the first two weeks of the postnatal period. It was shown that one of the causes of the failure of adaptation might be the disruption of the functional activity of the mitochondrial apparatus of brain cells mediated by the disruption of the first and second respiratory chain complexes. Exposure to chronic prenatal hypoxia has no significant effect on the increased risk of seizure activity in mice during audiogenic stimulation in late postnatal development, does not activate the development of persistent neurological deficit, and does not significantly affect the cognitive functions and learning ability of animals.

Keywords: prenatal hypoxia, mice, audiogenic seizures, neurological deficit, behavior, memory.

List of Abbreviations

GABA – γ-aminobutyric acid
CNS – central nervous system
PBS – phosphate-buffered saline

Introduction

Hypoxia is one of the most common stress factors included in the list of key triggers of CNS pathologies, including ischemic stroke, neurodegenerative and neurooncological processes. The need to consume large amounts of oxygen for the implementation of its functions as well as a limited set of antioxidant enzymes and compensatory capabilities makes the brain a central target organ for the negative effects of oxygen deficiency (Mitroshina *et al.*, 2021a; Radak *et al.*, 2017; Volpe, 2008; Miyamoto & Auer, 2000). The key consequences of triggering hypoxia-induced pathological mechanisms are mitochondrial dysfunction, loss of synapses and disruption of synaptic transmission, activation of inflammatory and apoptotic reactions that mediate the death of nerve cells, and the loss of functionally significant elements of neuron-glial networks (Choi, 2020; MacDougall *et al.*, 2019; Chen *et al.*, 2018; Mukandala *et al.*, 2016).

Due to the individual resistance of the human body to hypoxia and, in particular, the level of the adaptive reserve of the brain and the plasticity of the nervous tissue, it remains relevant to study the fundamental mechanisms of hypoxia and develop effective patient-oriented therapeutic strategies that activate protective and adaptive mechanisms in the cells of the nervous system when exposed to oxygen deficiency. Researchers and clinicians pay special attention to the problem of prenatal hypoxia. About 65% of diagnosed cases of CNS damage in newborns are caused by hypoxia-ischemic disorders, which increase the risk of infant mortality and severe disability (Piešová & Mach, 2020; Riljak *et al.*, 2016; Lawn J.E. *et al.*, 2005). Chronic prenatal hypoxia is the most severe form of

hypoxia that develops during pregnancy. In addition to the formation of gross malformations and congenital anomalies, neurological disorders, cognitive impairments, and delayed psycho-emotional development (Fisher *et al*., 2020; Nalivaeva *et al*., 2018; Giannopoulou *et al*., 2018; Rocha-Ferreira & Hristova, 2016), prenatal hypoxia can provoke the development of epileptiform activity (De Haan *et al*., 2018; Zhuravin *et al*., Kalinina *et al*., 2015; Turovsky *et al*., 2013; Hossain, 2005). It is assumed that in the case of prenatal hypoxia, the development of epileptiform activity in the fetus occurs due to changes in the balance of excitatory and inhibitory processes, which, in turn, contribute to the development of neuronal synchronization manifested in the spontaneous rhythm of electrical activity, and the formation of seizure activity (Kobylarek 2019; Kalinina *et al*., 2015, 2019; Glass, 2014; Fumagalli *et al*., 2009). However, the true causes of epilepsy and the role of prenatal hypoxia in the development of this pathological process remain poorly understood.

Herein, we characterized the functional state of mice exposed to chronic prenatal hypoxia during the first three postnatal weeks and assessed the risks of developing epileptiform activity when provoking audiogenic seizures.

Materials and Methods

Research object

Hybrid mice of two lines C3H and C57Bl6 (C3H+C57Bl6) obtained by the crossing scheme shown in Fig. 1 were used in this study. The obtained line was propagated and housed in a certified SPF vivarium of the Lobachevsky State University of Nizhny Novgorod. All experimental procedures were approved by the Bioethics Committee of Lobachevsky University and carried out in accordance with Act 708n (23 082010) of the Russian Federation National Ministry of Public Health, which states the rules of laboratory practice for the care and use of laboratory animals, and the Council Directive 2010/63 EU of the European Parliament (22 September 2010) on the protection of animals used for scientific purposes.

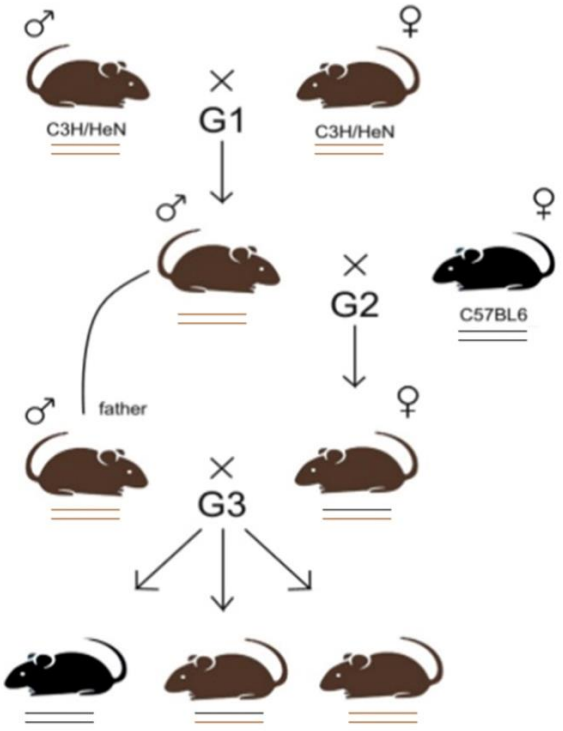

Fig. 1. Scheme of obtaining a hybrid line of mice

Scheme of the experiment

Pregnant females of the hybrid line of mice were simulated with chronic prenatal hypoxia from day 14 of gestation until delivery. A control group consists of pregnant females not subjected to hypoxia modeling. The functional activity of brain mitochondria was assessed in the newborns at the P0 stage. During the first three postnatal weeks, the height and weight characteristics of the offspring were measured. On the 21st day, animals were provoked to audiogenic seizures, followed by the evaluation of neurological status, general motor and orienting-exploratory activity in the Open Field test; mnestic and cognitive functions were assessed in the test of conditioned passive avoidance reflex (CPAR).

Chronic prenatal hypoxia model in vivo

Simulation of chronic prenatal hypoxia was carried out by daily placement of pregnant females in a vacuum flow-type chamber, in which a pressure of 177–218 mm Hg (≈ 6500–7000 m above sea level) was maintained for 2 hours (Shchelchkova *et al.*, 2020; Urazov *et al.*, 2018). The outside air temperature was 20–

–22 °C. The ascent to the simulated height was performed from the 14th day of gestation until delivery.

Assessment of the functional activity of brain mitochondria

Functional activity of brain mitochondria was assessed in newborn mice at the stage of P0. Brain mitochondria were isolated using the standard differential centrifugation method (Novozhilova *et al.*, 2021; Astrakhanova *et al.*, 2018; Pallotti & Lenaz, 2001). All manipulations were performed on ice using ice-cold reagents. The newborns were decapitated, and the brains without cerebellum were quickly isolated and placed in the porcelain mortar. The brain tissue was homogenized in an isolation medium (210 mM mannitol, 70 mM saccharose, 10 mM HEPES, 0.1 mM EDTA (pH 7.4)) using the glass homogenizer. After the double centrifugation (1100 g and 8500 g at 0°C) of the brain homogenate, the isolated mitochondria were placed in an incubation medium (120 mM KCl, 5 мМ NaH_2PO_4, 10 mM HEPES, 5 mM glutamate, 5 mM malate, and 14 mM MgCl2 (pH 7.4)). The registration of the oxygen consumption by the mitochondria was performed using a high-resolution respirometer Oxygraph-2k (Oroboros, Austria) in a closed chamber at constant stirring and controlled temperature (37 °C). The concentration of mitochondrial protein in a chamber measured by the Bradford method was 0.5 mg/ml.

The following parameters of the functional state of the mitochondrial respiratory chain were assessed: 1) V4 – the rate of oxygen consumption by the mitochondria at high content of substrates 5 mM glutamate and 5 mM malate (substrates of complex I); 2) V3 – oxidative phosphorylation rate in V4 conditions supplemented with 5 mM adenosine diphosphate (ADP). The intensity of complex II of the respiratory chain was assessed after complex I inhibition by 0.5 μM rotenone and complex II stimulation with 10 mM sodium succinate.

Provocation of audiogenic seizures

The provocation of audiogenic seizures in small laboratory animals in response to sound stimulation is considered one of the generally accepted experimental models of human generalized convulsive epilepsy (Jobe & Browning, 2006). On day 21 of the postnatal period, audiogenic seizures were provoked in mice in accordance with the study of Semiokhina et al. (Semiokhina *et al.*, 2006). Audiogenic seizures were simulated using a Startle and fear condition (PanLab, Spain; Stoelting, USA) placed in a soundproof box. The animal was placed in the cage, and after a 1-minute adaptation, a single electromechanical bell with a sound intensity of 110 dB was given. The sound signal was turned off immediately after the onset of a seizure or after 1 minute after the bell was turned on. Video recording of behavioral reactions was carried out using a Microsoft LifeCam Cinema HD camera.

The intensity of seizure activity manifestation in response to sound stimulation was assessed according to the Krushinsky scale:

0 points – no response to sound for 1 minute;

1 point – the phase of "manege running" or motor excitation – the animal makes uncontrolled movements in the cage after the start of the sound signal;

2 points – the beginning of clonic seizures with the animal falling on its belly (the beginning of seizures);

3 points – falling on the side, clonic seizures of the fore and hind limbs;

4 points – tonic seizures of the forelimbs, clonus of the hind limbs;

5 points – tonic seizures of the fore and hind limbs, accompanied by the rigidity of the entire body of the animal (Borisova *et al.*, 2018; Krushinsky *et al.*, 1948).

In addition to assessing the intensity of the seizures, the number of fatalities was also assessed.

Neurological status assessment

The analysis of the development of neurological deficits in animals was carried out according to the Scale for the Assessment of Neurological Deficits in Small Laboratory Animals with modifications (Novozhilova *et al.*, 2020; Beni-Adani *et al.*, 2001). In each animal, 10 involuntary innate behavioral responses were rec-

orded, each of which was assessed by a scoring system. The scale includes 10 tests to identify features of motor activity, trajectory and coordination of movements, the severity of reflexes, muscle tone, presence/absence of ptosis and exophthalmos. If the animal performed the test, the score is 0 points; performed partially – 1 point; if no reaction was observed – 2 points. Based on the test results, the scores were summarized and interpreted according to the following gradation: 10–20 points – severe CNS injury; 6–9 points – moderate CNS damage; 1–5 points – slight CNS damage.

Open field test

The study of the general motor and orienting-exploratory activity of the animals was performed under Open field conditions. Video recording of the animal's behavioral reactions was carried out using a Sony SSC-G118 video camera (Japan) for 5 min. The following main behavioral responses were analyzed: the number of crossed squares, vertical motor activity with fixation of the total number of upright postures, the number of acts of grooming, defecation, urination, and time spent in the center of the arena, which characterizes the emotional state of the animal.

Test of conditioned passive avoidance reflex (CPAR)

A chamber (60×20×25 cm) with an electrified slatted floor, divided by a partition into darkened and lighted compartments (Shuttle Box LE918; Panlab Harvard Apparatus, Spain) was used to study the ability of animals to learn. The training was performed the day after the provocation of audiogenic seizures. The animal was placed in the illuminated compartment, and the latent period of transition to the dark compartment was measured. After the animal entered the dark compartment of the chamber, an electrical impulse (0.08 mA) was applied for 5 s as a stimulus. Twenty-four hours later, a second test was performed to assess the time of transition to the dark compartment. The duration of the first training and repeated testing was 180 s.

Statistical analysis

Data are presented as the Mean ± standard error of the mean (SEM). Statistical analyses were performed on GraphPad Prism (v.6.0). Differences between groups were considered significant if the corresponding p-value was less than 0.05.

Results

The studies showed that exposure to chronic prenatal hypoxia had no effect on the number of animals in the litter; however, it led to a decrease in the survival rate of individuals in the first two postnatal weeks (Table 1). Mortality in the "Hypoxia" group was 8%.

The mitochondrial apparatus is the cell compartment most sensitive to the effects of hypoxia (Odorcyk *et al.*, 2021). In this regard, we evaluated the functional activity of mitochondria in the brain cells of newborn mice at the P0 stage (Fig. 2). The study showed that basal mitochondrial oxygen consumption rate during the oxidation of glutamate and malate substrates in the "Hypoxia" group had no significant differences from the "Control" group (Fig. 2A). Nevertheless, despite preserving the basal rate of mitochondrial oxygen consumption, the impact of chronic prenatal hypoxia leads to significant disruptions in the functioning of the first and second complexes of the mitochondrial respiratory chain. Thus, when the first complex of the respiratory chain was stimulated with ADP (Fig. 2B), the values in the "Hypoxia" group averaged 21.71 ± 9.89 pmol/(s*mL), which is 14% lower than in the "Control" group (148 ± ± 33.33 pmol/(s*mL)). Under conditions of first complex inhibition, the values in the "Hypoxia" group (7.86 ± 5.43 pmol/(s*mL) did not have significant differences relative to those in the "Control" group (7 ± 1.85 pmol/(s*mL)) ($p > 0.05$, the Mann-Whitney test). However, when the first complex of the respiratory chain was stimulated, there was no activation of the alternative succinate-dependent respiratory pathway (Fig. 2C), with values in the "Hypoxia" group (20.14 ± 8.68 pmol/(s*mL)) being on average 20% lower than those of the "Control" group (100.5 ± 17.52 pmol/(s*mL)). An assess-

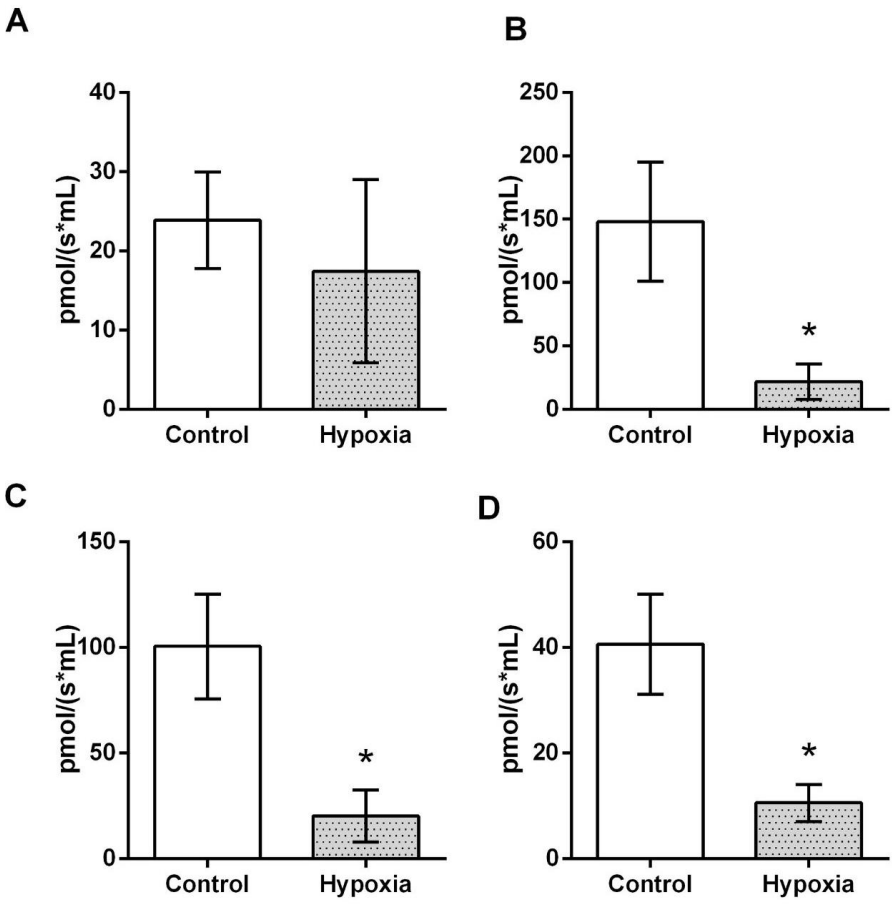

Fig. 2. Functional activity of the mitochondrial apparatus of brain cells of newborn mice exposed to chronic prenatal hypoxia. A – basal rate of oxygen consumption by mitochondria; B – ADP-stimulated respiration; C – activation of the alternative pathway of the respiratory chain; D – proton leak. Data are presented in recalculation per 1 mg of protein. * – *vs.* Control, the Mann–Whitney test

ment of the magnitude of the proton-driving force which not involved in the process of respiration (Fig. 2 D) revealed a 26% decrease in the proton leak rate in the "Hypoxia" group (10.57 ± ± 2.49 pmol/(s*mL)), on average, relative to control values (40.63 ± 6.73 pmol/(s*mL)).

When analyzing the weight and height characteristics, it was shown that during the first three weeks of the postnatal period, the mice of the "Control" group and the survivors of the experimental group gradually gained height and weight. However, according to the analyzed parameters, the offspring exposed to chronic prenatal hypoxia showed developmental delay. In particular, the body length of individuals obtained from hypoxia-induced females on the first day after birth averaged 4.31 ± 0.05 cm, which was significantly less than in the "Control" group (4.57 ± 0.04 cm). By day 21 of the postnatal period, the height parameters of the animals of the "Hypoxia" group remained reduced relative to control individuals. In contrast, females' weight and height characteristics were lower than males (Table 1).

On day 21 of the postnatal period, the mice were exposed to audiogenic seizures modelling. The studies showed that stimulation of chronic prenatal hypoxia does not provoke the development of epileptiform activity in offspring. In the animals of the control and experimental groups, the intensity of seizure activity in response to audiogenic stimulation was 0 points (no response to sound for 1 minute) according to the Krushinsky scale in 100% of cases.

Table 1

Body mass and body length values of newborn mice exposed to chronic prenatal hypoxia during three weeks of the postnatal period

Day of postnatal period	Control		Hypoxia	
	Body weight, g	Body length, cm	Body weight, g	Body length, cm
P1	1.62 ± 0.03	4.57 ± 0.04	1.65 ± 0.04	**4.31 ± 0.05***
P7	4.83 ± 0.23	7.58 ± 0.12	4.95 ± 0.12	7.47 ± 0.08
P14	8.20 ± 0.24	11.24 ± 0.16	8.11 ± 0.26	11.17 ± 0.09
P21 (♂+♀)	*10.8 ± 0.29*	*14.04 ± 0.14*	*11.12 ± 0.36*	***13.51±0.10****
P21♂	10.89±0.32	14.02 ± 0.15	**11.90** ± **0.44*#**	**13.73** ± **0.11#**
P21♀	10.69±0.56	14.08 ± 0.25	10.27 ± 0.49	**13.27** ± **0.14***
Average number of pups in a litter	8.3		8.3	
Mortality in the first two weeks after birth, %	0		8	

Note: the data are presented as the Mean ± standard error of the mean (SEM). * – vs «Control», # – vs females (P21) within the group, $p < 0.05$, Student's t-test

Table 2

Parameters of behavioral reactions of mice in the Open Field test after provocation of audiogenic seizures

Parameters	Control		Hypoxia	
	♂	♀	♂	♀
Total passed distance, cm	1136.28 ± 82.4 #	787.53 ± 81.81	695.30 ± 87.85 *	811.75 ± 133.80
Total distance passed in the arena center, cm	121.01 ± 24.41 #	54.03 ± 10.34	58.68 ± 12.23 *	62.35 ± 21.13
Total distance passed in the periphery of the arena, cm	1015.23 ± 69.82 #	733.50 ± 77.88	636.63 ± 81.86 *	749.39 ± 122.86
Time spent in the arena center, s	20.38 ± 6.96	10.51 ± 3.42	15.93 ± 4.59	9.9 ± 3.68
Time spent in the periphery of the arena, s	279.62 ± 6.96	289.49 ± 3.42	284.07 ± 4.59	290.05 ± 3.68
Number of upright postures	30.15 ± 5.10 #	15.27 ± 3.02	7.75 ± 1.94 *	15.00 ± 4.08
Acts of grooming	1.61 ± 0.14 #	2.09 ± 0.16	1.42 ± 0.15	2.00 ± 0.27
Acts of urination	0.615 ± 0.21	0.82 ± 0.23	0.92 ± 0.26	0.45 ± 0.16
Acts of defecation	3.15 ± 0.54	3.18 ± 0.44	2.42 ± 0.64	2.64 ± 0.43

Note: the data are presented as the Mean ± standard error of the mean (SEM). * – *vs.* "Control", # – *vs.* females (P21) within the group, $p < 0.05$, ANOVA with Tukey post hoc multiple comparison test

The following day after the provocation of audiogenic seizures, the neurological status, and motor and orienting-exploratory activity of mice in the Open Field test were evaluated.

An assessment of the neurological status of mice using the scale of severity of neurological deficit did not reveal pronounced CNS damage in the control and experimental groups of ani-

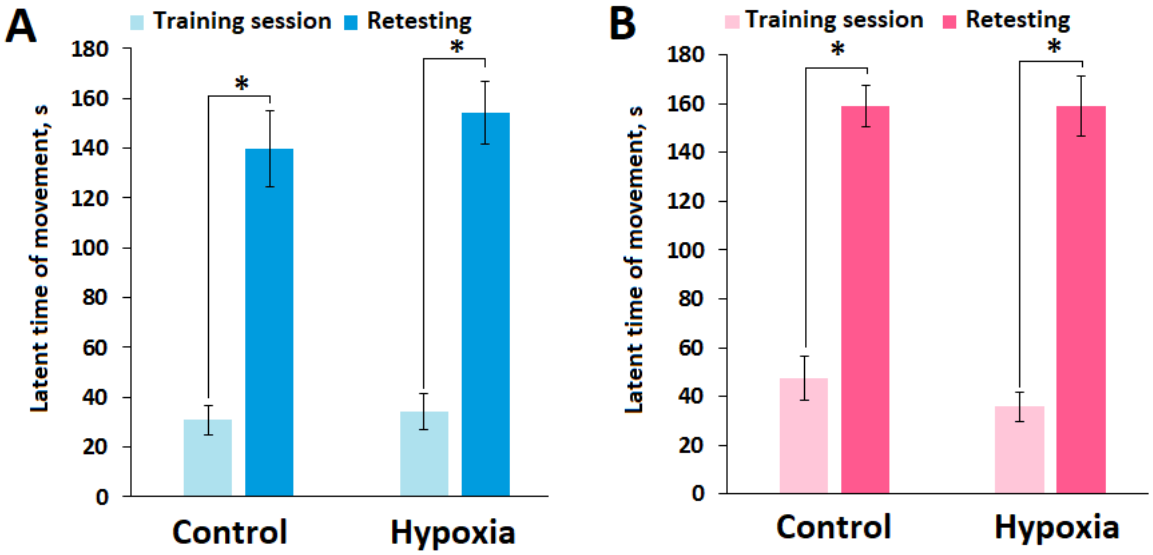

Fig. 3. The efficiency of reproduction of the conditioned passive avoidance reflex in mice after the provocation of audiogenic seizures.
* – *vs* "Training session", $p < 0.05$, ANOVA with Tukey post hoc multiple comparison test

mals. The values of neurological deficit in the "Control" and the "Hypoxia" groups for males were 2.3 ± 0.5 and 2.7 ± 0.4 points, for females – 2.5 ± 0.4 and 3.1 ± 0.5, respectively; no significant differences between the groups were found ($p > 0.05$, the Mann-Whitney test).

The results of the Open Field test (Table 2) showed that in response to the impact of audiogenic convulsions, males of the "Control" group showed greater than females motor activity both in the center and on the periphery of the arena; they also had increased research activity, characterized by an average 1.9-fold increase in the number of upright postures. Moreover, changes in the emotional state manifested in a decrease in the number of grooming acts were observed in males.

Interestingly, no pronounced changes in behavioral reactions were found between females and males of the "Hypoxia" group. Compared with the "Control" group, the males of the "Hypoxia" group had reduced motor and orienting-exploratory activity.

To assess cognitive functions and learning ability, the animals were tested to develop a conditioned passive avoidance reflex. The data reflecting the duration of the latent period of the transition between the light and dark chambers are presented in Fig. 3. The evaluation of these parameters did not reveal significant changes in the processes of memory formation and learning abilities in hypoxia-induced animals. At the stage of reproduction, the duration of the latent period in the "Hypoxia" group significantly increased relative to the parameters of the training stage on average by 4.5 times and did not differ from the values of the "Control" group. The delay in the motor response and the choice of a safe compartment indicates the normal physiological process of learning and the formation of a memory trace in the animal.

Discussion

Chronic prenatal and early neonatal hypoxia are the most severe forms of hypoxia, the timely diagnosis and effective treatment of which is an acute issue for neonatology. Current adverse environmental factors, maternal stress, smoking and alcoholism lead to insufficient oxygen supply to the fetus (Wang *et al.*, 2021; Piešová M & Mach M., 2020; Gumusoglu *et al.*, 2020). The brain is a central target organ for the damaging effects of hypoxia because of its need to consume large amounts of oxygen to maintain its metabolic and functional activity, as well as a limited set of antioxidant enzymes and com-

pensatory capabilities (Mitroshina *et al.*, 2021). In prenatal ontogenesis, hypoxia is among the main causes of disturbances in the formation of fetal brain structures and functions, which, in turn, predisposes to the development of neurological disorders, activation of neurodegenerative processes, and oncology (Wang *et al.*, 2021; Nalivaeva *et al.*, 2018; Giannopoulou *et al.*, 2018). A number of experimental and clinical studies showed that hypoxic conditions, including prenatal hypoxia, provoke the development of epileptiform activity (Zhuravin *et al.*, 2019; De Haan *et al.*, 2018; Kalinina *et al.*, 2015, 2019; Turovsky *et al.*, 2013, Hossain *et al.*, 2005). The brain's normal functioning depends on the timely development of the main excitatory and inhibitory components. Early neuronal synaptic responses to γ-aminobutyric acid (GABA) are excitatory; GABA becomes the main inhibitory neurotransmitter only in the early postnatal period (Farrant & Kaila, 2007). Untimely development of inhibitory tone probably contributes to a significant decrease in the sensitivity threshold and the onset of seizures (Kobylarek *et al.*, 2019; Karyakin *et al.*, 2017; Jantzie *et al.*, 2015; Fumagalli *et al.*, 2009). A second putative cause of epilepsy in the postnatal period may be hypoxia-induced oxidative stress (Kobylarek, 2019).

Taking into account the variety of causes of fetal hypoxia, the duration and intensity of hypoxic exposure, there remains a need to study the pathogenetic aspects of hypoxia, its role in the development of seizure activity, and ways to activate the adaptive capabilities of the CNS to conditions of oxygen deficiency.

In the present study, we assessed the role of chronic prenatal hypoxia on the functional state of C3H+C57Bl6 hybrid line mice during the first three weeks of postnatal development, and the risks of developing epileptiform activity when provoking audiogenic seizures.

It is currently known that the level of fetal adaptation to hypoxic damage and the severity of the negative consequences of prenatal hypoxia depend on the ontogeny periods in which oxygen supply was disturbed (Nalivaeva *et al.*, 2018). Exposure to hypoxia in the I-II trimester of pregnancy disrupts the process of fetal formation and increases the frequency of chromosomal aberrations; it mediates gross malformations and dysfunction of organs and systems of the whole body, which significantly increases the risk of fetal death. The consequences of hypoxia occurring at later stages of pregnancy (III trimester) are more associated with developmental disorders and alterations in brain functions in the postnatal period (Shchelchkova *et al.*, 2020; Urazov *et al.*, 2018; Otellin *et al.*, 2012).

In our studies, simulation of chronic prenatal hypoxia was performed from day 14 of gestation to delivery, which characterizes the II-III trimester of pregnancy. It has been shown that simulated stress maintains the risk of neonatal mortality and leads to hypotrophy of surviving individuals in the first week of postnatal development, primarily mediated by reduced height characteristics of individuals relative to the control group. We also observed gender specificity of morphometric parameters of hypoxia-induced animals. The weight and height characteristics of the females of the “Hypoxia” group were lower than the parameters recorded in the males of the same group on day 21 of postnatal development. The results obtained indicate not only individual but also gender differences in the resistance to the effects of chronic prenatal hypoxia, in particular, due to the level of the adaptive reserve of the brain and plasticity of the nervous tissue (Netto *et al.*, 2017; Sanches *et al.*, 2015).

It is known that the body's response to hypoxia includes various adaptive mechanisms that contribute to the elimination of hypoxia-induced functional and metabolic disorders, primarily aimed at maintaining mitochondrial function and enhancing nonspecific resistance to oxygen deficiency.

We showed that chronic prenatal hypoxia leads to significant impairments in the functional activity of the mitochondrial apparatus of the brain cells of newborn mice. Despite the preservation of the basal rate of oxygen consumption by the mitochondria of the brain cells of the “Hypoxia” group, low efficiency of the first and second complex of the mitochondrial respiratory chains during their stimulation was

observed. The observed changes can lead to the disruption of the body's adaptation to hypoxic damage. The development of mitochondrial dysfunction can lead to a decrease in ATP production, as well as an increase in the production of reactive oxygen species, which can provoke further disruption of the respiratory chain and, consequently, lead to profound suppression of the bioenergetic functions of mitochondria and cell death (Zhang *et al.*, 2021; Ten *et al*., 2021, Belosludtsev *et al.*, 2020). Nevertheless, the maintenance of the basal rates of oxygen consumption, which we observed in our study, may indicate the activation of the adaptive mechanisms of nerve cells due to an increase in the number of mitochondria (Fuhrmann & Brune, 2017). In addition, since mitochondrial respiration activity is maintained at control levels under conditions of inhibition of the first complex, it can be assumed that a possible way to maintain the basal rate of mitochondrial oxygen consumption in chronic oxygen deficiency may be the activation of alternative pathways for cytoplasmic NAD-H reoxidation (Ten *et al.*, 2021, 2022).

No less interesting is the study of the role of chronic prenatal hypoxia in the development of epileptiform activity in mice in the postnatal period of development. We showed that hybrid mice exposed to chronic prenatal hypoxia are highly resistant to the development of seizure activity in response to audiogenic stimulation. The animals in the “Hypoxia” group had no reaction to the sound signal (according to the Krushinsky scale). After the simulated stress, no neurological deficit or pronounced changes in the basic behavioral responses of both females and males were observed in the mice. The CPAR test also did not reveal significant changes in the processes of memory formation and learning abilities in hypoxia-induced animals. It can be assumed that exposure of hybrid line mice to chronic prenatal hypoxia does not cause the development of gross abnormalities in brain tissue morphology and the functional architectonics of neuron-glial networks, thereby retaining the potential for the development of long-term mechanisms of adaptation and synaptic plasticity of the nervous tissue, which determine resistance to simulated stress in late postnatal development. Histological analysis of the brain tissue of the hybrid line of mice subjected to chronic prenatal hypoxia in the early and late postnatal period will shed light on this issue and is the aim of our further research. A detailed characterization of the phenotypic status of the hybrid line will allow us to describe in more detail the mutant mouse lines created by ENU mutagenesis and evaluate the role of chronic prenatal hypoxia in provoking seizure activity in individuals with a genetic predisposition to epilepsy.

Conclusion

Exposure to chronic prenatal hypoxia increases the risk of neonatal mortality and developmental delay in survivors in the first two postnatal weeks. One of the causes of disruption of adaptation can be impaired functional activity of the mitochondrial apparatus of brain cells, primarily mediated by disruption of the first and second respiratory chain complexes. Exposure to chronic prenatal hypoxia does not significantly increase the risk of seizure activity in mice with audiogenic stimulation in late postnatal development, does not subsequently activate the development of persistent neurological deficit, and does not significantly affect the cognitive functions and learning ability of the animals.

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