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Ontogenetic Characteristics of Behavior in Rats Subjected to Hypoxia on Day 14 or Day 18 of Embryogenesis

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Physiological development, motor activity, and cognitive functions were studied in rats subjected to acute normobaric hypoxic hypoxia (3 h at an O_2 concentration of 7%) at different stages of embryogenesis (days E14 or E18). Prenatal hypoxia was found to lead to delays in physiological development and the establishment of motor behavior during the first month of postnatal ontogenesis. These changes were more marked in rats subjected to hypoxia on day 14 of intrauterine development and disappeared with age. In adult rats, regardless of the timing of exposure to hypoxia (E14 or E18), learning ability was degraded and long-term and short-term memory were impaired. These results suggest that exposure to the pathogenic factor during the main period of neuroblast generation and migration (E14) was significant both for physiological development and the establishment of motor behavior in the animals and for the execution of the cognitive functions of the brain, while exposure during the period at which maturation and differentiation processes dominate in the brain (E18) was more significant in relation to the execution of cognitive functions.

Key words: ontogenesis, prenatal hypoxia, behavior, learning, memory, cognitive functions, rats.

Hypoxia impairs the functional activity of many body systems, primarily affecting the central nervous system [18, 22]. Pathological changes associated with oxygen insufficiency most frequently affect the cerebral cortex, basal ganglia, hippocampus, and cerebellum [1, 13, 27]. All of these brain structures are directly associated with the organization of behavior in animals, including both simple innate and more complex (acquired during training) motor acts. The most serious and multifarious sequelae of hypoxia appear during intrauterine development, when the rudiments of the growing body's organs are laid down. There are particular periods of embryogenesis during which the actions of the pathological factor lead to severe nervous system impairments [4, 6, 8]. This is particularly associated with heterochrony in the formation of nerve cells, which become more susceptible at the moment of DNA replication, when the probability of genetic lesions increases. Periods of cell proliferation differ [9] depending on their type and association with particular brain structures (Fig. 1). Brain cell proliferative activity during embryogenesis is also heterogeneous, for example reaching a peak at the end of the second week of intrauterine development and then decreasing in rats. The decrease in proliferative activity is characterized by an increase in the duration of the mitotic cell cycle [9] and a reduction in the number of cells in mitosis [19]. Thus, we suggested that hypoxia at the early stages of embryonic development might act on the rudiments of particular brain cell populations (Fig. 1), leading to fundamentally different structural-functional abnormalities in the CNS, resulting in changes in different types of behavior and the execution of cognitive functions in later ontogenesis. The aim of the present work was to investigate the establishment of motor behavior and the execution of cognitive functions after acute hypoxia on day 14 of embryogenesis in rats, when the brain is dominated by proliferation and migration processes, as compared with hypoxia on day 18 of embryogenesis, when nerve cell differentiation processes start to become dominant.

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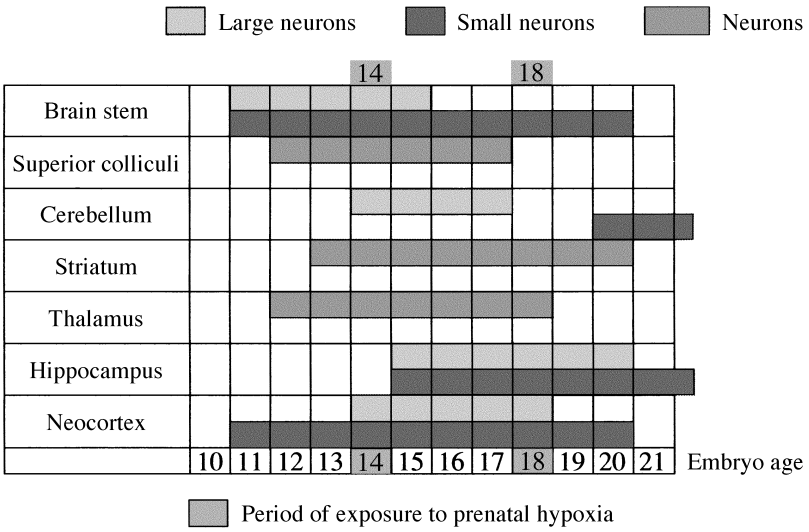

Fig. 1. Periods of cell generation in different parts of the brain [9].

METHODS

Studies were performed on Wistar rats reared at the Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, in accordance with the "Regulations for Studies using experimental Animals." Offspring meeting the requirements of the experiments were obtained by placing virgin females aged 3–4 months in cages with males in pairs in animal-house cages. The timing of fertilization was monitored using vaginal smears. The day on which spermatozoids were seen on smears was taken as the first day of pregnancy. Fertilized females (a total 64) were divided into groups of 4–5 individuals in animal-house cages in a room with normal illumination and a constant temperature of 20–23°C with free access to water and food. On days 14 or 18 of pregnancy, some of the females were subjected to hypoxia in a special chamber of volume 100 liters fitted with a temperature control system, ventilation, a system for gas analysis, and a system for adsorption of exhaled CO_2. During experiments, the oxygen content in the chamber was decreased from 20.7% to 7.0% and maintained at this level for 3 h. The carbon dioxide concentration in the chamber was no greater than 0.2% and the temperature was maintained at 22°C. Animals of the control group were kept for 3 h in the same conditions with a normal O_2 concentration. No more than 10 rats were placed in the chamber simultaneously. On day 20 of pregnancy (one day before birth), females were placed in individual cages. Eight rat pups remained in each litter on postnatal day 2. When calculating the ages of rat pups, the day of birth was taken as day zero. At age 30 days, rat pups were separated from their mothers and placed in cages in groups of 4–5 individuals of the same gender.

The pups' body weight was measured daily during the first month from the moment of birth and the major developmental stages were monitored (eye opening time and the onset of separation of the external ear from the skin of the head). Body weight was again measured at age four months.

The rats' motor activity was studied in the control (n = 197) and experimental (hypoxia on days 14 (GE14, n = 214) and 18 (GE18, n = 151) of embryogenesis) using accepted current methods for testing tonic postural reactions [11, 30] and locomotion. Testing was performed daily at 14:00.

Forelimb placing reaction (days 1 to 21). Rats were taken by the tail and the whisker area was brought to the sharp end of a horizontally oriented pencil. The forelimb placing reaction was assessed for 1 min on a four-point scale: 0 corresponded to the absence of a reaction, 1 to a weak, chaotic elevation of the limb without making contact with the pencil, 2 to rotatory movements of the head and elevation of the limb to the support, and 3 to extension of the snout in the direction of the pencil with accurate and precise lifting of both forelimbs onto the support.

Negative geotropism (days 1–15). Rat pups were placed on an inclined, roughened surface (slope 20°, size 300 × 330 mm) with the head downward and the extent of rotation of the animal to reach the normal head upward position was monitored for 1 min. Results were assessed on a four-point scale, where 0 points corresponded to no reaction, 1 to slight rotation (up to 90°), 2 to incomplete rotation (more than 90°), and 3 to complete rotation (180°).

Rotation of the body from the back onto the abdomen (days 1–15). Rat pups were placed on their backs and reactions consisting of rotation of the body onto the abdomen were monitored for 1 min. Reactions were assessed on a

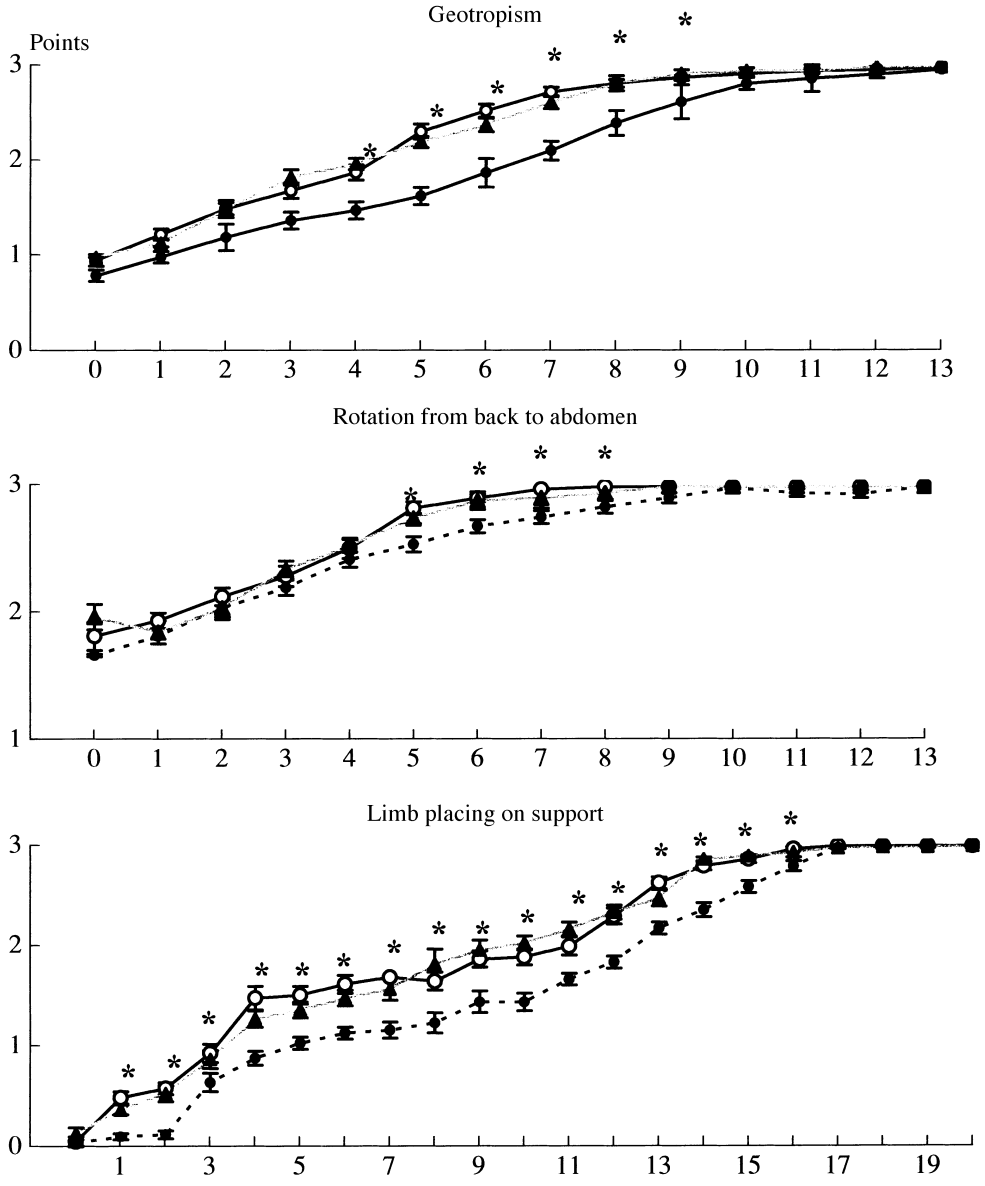

Fig. 2. Differences in the development of motor activity between control rats and animals subjected to prenatal hypoxia on day E14. White circles show the control group; black circles show the GE14 group; triangles show the GE18 group. Mean values ± errors of the mean are shown for each group. *Differences between the control and GE14 groups ($p < 0.01$).

four-point scale, where 0 points corresponded to the absence of any reaction and 1 to slight, 2 to incomplete, and 3 to complete rotation.

Maintenance on rotating grid (days 1–15). Rat pups were placed on a grid (100 × 100 mm, cell size 5 × 5 mm, filament diameter 1 mm) positioned horizontally and the angle of rotation of the grid, which moved at a rate of 2 rpm, was monitored for 1 min until the rat fell onto a soft foam mat.

Maintenance of hanging by the forepaws from a horizontal wire (days 7–17). Rat pups were brought to a wire (diameter 1 mm, length 145 mm) fixed horizontally above a soft foam mat at a height of 170 mm. When the rat clung to the wire and could hang by the forepaws independently, a stopwatch was started and the period of time during which the animals remained on the wire was measured (maximum 1 min).

Maintenance of balance on a rotating bar (days 14–30). The rat pup was placed (against the direction of rotation) on a rotating wooden bar (diameter 50 mm, length 195 mm, rotation rate 6 rpm) positioned horizontally above a foam mat at a height of 190 mm and the period during which the animal remained on the bar was measured (maximum 1 min).

TABLE 1. Changes in Mean Duration of Pushing a Piston after Training and after an Interval

Group	Control	GE14	GE18
Before training	33.36 ± 0.55	32.35 ± 1.19	32.37 ± 0.71
After training	49.49 ± 1.05*	40.11 ± 0.84*	42.03 ± 0.74*
Before the break	51.97 ± 2.08*	34.45 ± 1.11	31.78 ± 0.78

Note. *Statistically significant differences in the mean duration of application of pressure to a piston as compared with baseline (pre-training), $p < 0.001$.

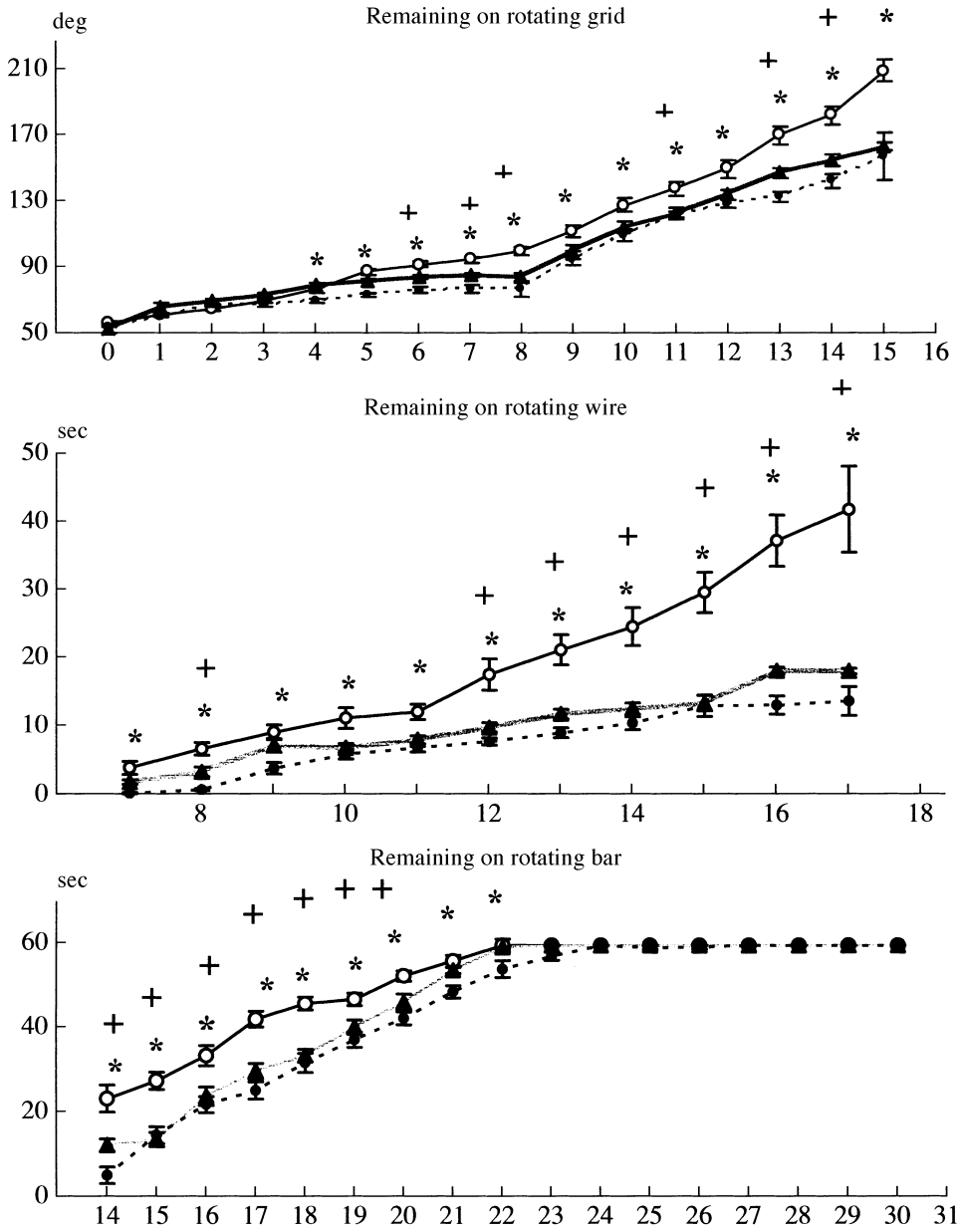

Fig. 3. Differences in the development of motor activity between control rats and animals subject to prenatal hypoxia on days E14 or E18. White circles show the control group; black circles show the GE14 group; triangles show the GE18 group. The abscissa shows the rats' age, days. Mean values ± errors of the mean are shown for each age. *Differences between the control and GE14 groups ($p < 0.01$); +differences between the control and GE18 groups ($p < 0.01$).

Locomotion in the open field. Rat pups (days 13–30) and adults (four months) were tested in open fields (500 × × 500 × 10 mm and 1000 × 1000 × 400 mm, respectively, divided into 25 equal parts) for 1 min and 10 min, respectively, measuring the number of squares crossed during excursions. The floor of the chamber was washed with 50% ethanol solution after each animal.

Training to an operant reflex in three-month-old male rats pups of the control (n = 13), GE14 (n = 14), and GE18 (n = 16) groups was performed in a chamber fitted with an automatic feeder and instruments for automatic recording of movements and delivery of reinforcement. Every day during two cycles of 64 movements each, the rats were trained to use the forelimb to press a piston attached horizontally to the front wall of the experimental chamber. Each press accompanied by holding the piston for more than a specified time period (usually 50 msec) was reinforced. The learning criterion was a statistically significant ($p < 0.01$) change in the mean duration of pressure application during seven training cycles (see [15] for more detail).

Eight-arm radial maze. One- and two-level mazes (Buresh et al., *Methods and Basic Experiments for Studies of the Brain and Behavior*, 1991) were used to record the numbers of correct, reinforced excursions (single visits to the maze arms) during one test cycle, which ended when the animal performed any eight excursions; the percentage of correct maze arm visits was calculated. Each group of rats, consisting only of males (control, n = 20; GE14, n = 17; GE18, n = 28) was tested 15 times in both mazes.

Analysis of results was performed using Microsoft Excel for Windows 98 and SigmaStat 3.0. Data were analyzed using descriptive statistics to assess arithmetic means and errors of arithmetic means. The hypothesis that means were equal in cases with normal distributions was tested using Student's test. The non-parametric Mann–Whitney–Wilcoxon test was used in other cases. Assessment of qualitative characteristics was performed using Fisher's test. Differences were regarded as statistically significant at the 5% significance level.

RESULTS

Assessment of the Overall Physiological Development of Neonatal Rats

As compared with controls, rat pups of group GE14 showed delayed development. From the moment of birth and throughout the first month of postnatal development, these animals had lower body weights, later separation of the external ear from the skin of the head (3.14 ± 0.4 days, $p < 0.05$), and later eye opening (17.32 ± 0.2 days, $p < 0.01$) as compared with controls (2.29 ± 0.18 and 15.64 ± 0.41 days, respectively). The GE18 group showed no statistically significant delay – the external ear separated at 2.9 ± 0.24 days and the eyes opened at 16.64 ± 0.35 days. Body weight in neonates of the GE18 group (5.83 ± 0.07 g) was not different from that of controls (5.71 ± 0.06 g) but was greater than that in rats of group GE14 (4.99 ± 0.09 g, $p < 0.01$). However, starting from day 4 and continuing to day 30 of postnatal development, mean body weight of rats in the GE18 group was different from that in controls and was comparable with that in pups of the GE14 group.

Development of Motor Responses in Rat Pups during the First Month after Birth

Previous studies have shown that during the first month of postnatal development, animals of the GE14 group showed a significant delay compared with the control group in terms of the level of development of motor reactions in the series of tests used in the preliminary study [6]. In the "Geotropism," "Rotation from back to abdomen," "Limb placing" (Fig. 2), and "Open field locomotion" tests, mean values for motor activity in rat pups in the GE18 group were different ($p < 0.05$) from those in the GE14 group and were similar to those in control rats. At the same time, other tests reflecting the animals' ability to remain on a horizontal wire, rotating grid, and wooden bar showed that average values for motor activities in rats of the GE18 group were statistically significantly different from those in controls (Fig. 3), at certain times exceeding ($p < 0.05$) the average values for animals of the GE14 group. Thus, in terms of the development of motor activity, animals of the GE18 group generally differed from those of the GE14 group, but could be similar to or different from the control group, depending on the reaction being tested.

With time, the differences seen between rats subjected to prenatal hypoxia and control rats disappeared. Ault male rats had the same body weight (244.38 ± 3.44 g in GE14 rats and 247.24 ± 9.37 g in GE18 animals) as control rats (243.50 ± 6.28 g). By four months, rats of all the study groups demonstrated identical abilities to remain on the horizontal wire (35.95 ± 4.21 sec in control rats, compared with 30.70 ± 4.89 and 29.52 ± 2.81 sec in the GE14 and GE18 groups, respectively) and similar levels of motor activity (29.20 ± 4.63, 28.56 ± 4.19, and 30.52 ± 2.81 in the control, GE14, and GE18 groups, respectively).

LEARNING AND MEMORY

Our studies have previously demonstrated that exposure to hypoxia on day 14 of embryonic development leads to degradation of memory processes and the retention of learned operant skills in adult animals [6]. In the present study, the GE14 group, as compared with controls, showed a reduction in the number of rats showing good learning (Fig. 4), while the mean duration of application of pressure to the bar following initial training in these animals underwent significantly smaller changes, returning to baseline, i.e., to the pre-training level, after a break (Table 1). In the

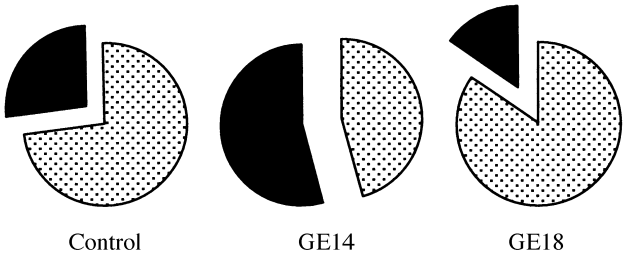

Fig. 4. Ability of adult rats of different groups (control, GE14, GE18) to learn an operant movement of the forelimb to apply pressure to a piston. The light segment shows the number of rats showing learning as a percentage of all rats showing learning; the dark segment shows rats not showing learning.

GE18 group, the number of adult rats showing good learning was not statistically significantly different from that in controls (despite the fact that it was 17% greater than the control value, Fig. 4). As compared with the GE14 group, the number of adult rats demonstrating good learning was 44% greater ($p < 0.05$) in the GE18 group. The mean duration of applying pressure to the blocking piston in adult animals following initial training changed by 26.49 ± 6.90% in the GE14 group and by 30.90 ± 4.47% in the GE18 group, which was statistically smaller ($p < 0.05$) than the changes seen after training the control animals (50.32 ± 6.46%).

Long-term memory following initial training was studied by introducing a five-week break. Testing of the animals after the break showed that the control group retained the acquired learning level, while rats of the GE18 and GE14 groups showed reductions in the mean duration of pressure application to the level recorded prior to training (Table 1). Thus, rats subjected to prenatal hypoxia lost the acquired operant skill.

Studies of short-term memory were performed in an eight-arm maze. No differences between groups were seen in the one-level maze. The development of stereotypical behavior, whereby the rat visited each arm in sequence in a given direction (to the right or left) was excluded using a two-level radial maze. These experiments showed that on the background of the absence of differences in the mean duration spent by the rats with arms between groups, the proportion of correct feeder visits (97.57 ± 0.84% in controls) was significantly decreased both in rats of the GE18 group (to 91.29 ± 1.34%, $p < 0.001$) and the GE14 group (89.69 ± 0.99%, $p < 0.01$).

DISCUSSION

The decrease in the body weight of rats subjected to prenatal hypoxia on day GE14 is entirely consistent with known restrictions to intrauterine growth and impairments to embryo development due to oxygen deficiency [22]. Embryo growth is known to depend on the normal functioning of the placenta and the ability of oxygen and nutrients to cross from the mother's body [24]. Impairments to this exchange between mother and fetus can lead to reductions in fetal body weight. This weight reduction may be an adaptive reaction of the embryo to restricted nutrient and oxygen supplies, associated with reductions in the rate of cell division, especially in tissues with high levels of proliferative activity and/or reactions to changes in the activity of growth factors, such as insulin and/or insulin-like growth factor 1 [12, 28].

Oxygen deficiency during the embryonic period leads to significant changes in subsequent ontogenesis, affecting the motor, emotional, cognitive, and social spheres, though data on this area are often contradictory. Thus, prenatal hypoxia in rats has been reported as being followed both by delays in the development of motor coordination [14, 15, 17] and by the absence of any changes in rotating the body from the back to the abdomen and the geotropism reaction [16]. Prenatal hypoxia elicits changes in emotionality in rats, which is apparent as increased vocalization on being startled [22] and increased anxiety accompanied by hyperactivity in the open field test [10]. However, data have also been obtained showing decreases in investigative activity in the open field test [14] or the absence of locomotor reactions to hypoxia [16]. Prenatal hypoxia leads to decreases in social contacts in rats [22] and reduced cognitive capacities [15, 22], impairments in male sexual behavior [16], degraded acquisition of passive and active avoidance reflexes in rats [2, 10], and deteriorations in orientation in a water maze [14]. Our data indicate that the characteristics and strengths of body reactions during subsequent ontogenesis show a significant level of dependence on the timing (E14, E18) of exposure to the pathogenic factor during prenatal development.

Neurological testing of animals is often used to assess the development and functional state of different levels of the CNS. The brainstem, cerebellum, and spinal cord are known to play important roles in controlling posture, though higher postural control is mediated by the motor cortex and basal ganglia. Our experiments have shown that rats subjected to hypoxia of days 13–14 of embryonic development and having impairments to the processes forming motor and tonic postural reactions showed delay in the maturation of nerve cells and increased neurodegeneration in the cortex and striatum at different stages of postnatal ontogenesis as compared with controls [1, 4, 6]. These structural changes reached a peak by 20–30 days, and were no longer apparent by the end of the second month of postnatal ontogenesis, though decreases in the number of synaptopodin-positive labile spines in rat brain nervous tissue persisted throughout ontogenesis [1], which may point to decreases in the plastic properties of nervous tissue with age.

In rats of the GE18 group, morphological changes in the cortex and striatum were not seen, while behavioral experiments showed that these animals were no different

from controls in a series of tests. At the same time, differences between behavioral measures in control rats and GE18 rats seen on testing the animals' ability to remain on the horizontal wire, the rotating grid, and the wooden bar were statistically significant but less marked than the differences between rats of the control and GE14 groups. Differences in the behavior of GE18 rats from control were seen in the tonic posture dynamic tests, whose performance requires significant muscular force. The delay in weight gain in rats of the GE18 group (as in the GE14 group) as compared with controls may be linked with delayed development of the muscle apparatus. The prolonged period of maturation of neuromuscular junctions may play a defining role in limiting development of motor behavior; during this maturation, there is a transition from multi-motoneuron innervation of a given muscle fiber to mono-motoneuron innervation, allowing gradual and synchronous recruitment of muscle fibers during the development of muscular force [20].

Furthermore, during the first month of postnatal ontogenesis, the cortex and striatum of rat pups subjected to hypoxia, as compared with controls, showed differences consisting of changes in the activity of both the soluble form of acetylcholinesterase [6], which has growth properties [29], and its membrane-bound form. It is of note that these differences characterizing the activity of the cholinergic system of the brain were greater in rat pups subjected to hypoxia on E14, which may have greater influences on the motor behavior of rats of the GE14 group.

Considering the critical role of the normal development of the cortex and striatum in executing the types of movement studied here, it is impossible not to note the function of the cerebellum – the production of large cerebellar cells begins simultaneously with the development of the active proliferation process in the cortex and striatum (Fig. 1). Oxygen deficiency during the intrauterine period is known to lead to structural damage to the cerebellum [27], whose development is directly linked with the establishment of such dynamic tonic postural reflexes as remaining on the horizontal wire and the rotating grid and bar, as mutant mice with Purkinje cell and granule cell degeneration demonstrated delays in these tests as compared with siblings with the normal genotype [30]. The behavioral changes observed here may therefore also be associated with impairments to cerebellar function due to prenatal hypoxia.

Unlike purely motor impairments, cognitive changes occurring as a result of prenatal hypoxia also persisted in adult rats. Regardless of the timing of hypoxia (E14 and E18), adult rats showed deterioration of the process of acquiring operant movements, along with impairments to long-term and short-term memory. The cognitive impairments seen in adult animals may be induced by neurodegenerative processes in the brain seen in rats aged up to two months [4, 6], as well as decreases in the density of labile spines at later periods of ontogenesis [1]. In addition, hypoxia is known to decrease brain tissue ganglioside contents [25], which may alter the normal process of establishment of connections between neurons [26] and lead to impairments to memory formation [7]. The impairments of the activity of the brain cholinergic system occurring after hypoxia [4, 22], noted above, may also lead to cognitive impairments, as the cholinergic system is the main substrate for learning and memory processes [23], and changes in the state of the cholinergic system of the sensorimotor cortex and striatum lead to impairments to the learning of new operant movements and their correct performance [5].

Our experiments also demonstrated that hypoxia leads to decreases in the activity of amyloid-degrading enzymes and α-secretase, involved in the non-pathogenic scission of the amyloid peptide precursor [21]. Administration of inhibitors of these metalloproteases (batimastat and phosphoramidon) led to memory impairments [3], which may indirectly explain memory impairments following prenatal hypoxia in terms of changes in metalloprotease concentrations, including those involved in amyloid peptide metabolism.

Thus, the results obtained here provide evidence that prenatal hypoxia leads to compensatory changes in motor reactions in rat pups and cognitive dysfunctions seen in adult animals. The effects of the pathogenic factor during the period dominated by neuroblast generation and migration (E14) are significant both for the physiological development and establishment of motor behavior in animals, but also for the execution of the brain's cognitive functions, while the period during which the brain is dominated by maturation and differentiation processes (E18) is more important for cognitive functions.

CONCLUSIONS

1. The offspring of rats subjected to 3-h normobaric hypoxia (7% O_2) during pregnancy on days 14 or 18, as compared with the offspring of control rats, showed delays in physiological development and the formation of motor behavior during early postnatal ontogenesis. These differences in the performances of rats of the control and experimental groups disappeared by adulthood.

2. Exposure to hypoxia on day 14 of intrauterine development was followed by changes in the rats' behavior in all the tests used during the first month of postnatal development. Rats exposed to hypoxia on day E18, as compared with controls, showed delay only in tonic postural dynamic tests, whose performance requires significant muscular force. The differences seen here were statistically significantly smaller than the differences seen between control rats and rats of the GE14 group.

3. Unlike motor abnormalities, cognitive impairments (degradation of the ability to learn an operant reflex and impairments to long-term and short-term memory) were seen in adult rats and were independent of the timing of exposure to hypoxia (E14 or E18).

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REFERENCES

1. D. S. Vasil'ev, N. L. Tumanova, and I. A. Zhuravin, "Structural changes in the nervous tissue of the neocortex during the ontogenesis of rats after hypoxia at different stages of embryogenesis," *Zh. Évolyuts. Biokhim. Fiziol.*, **44**, No. 3, 258–266 (2008).
2. L. A. Vatava, V. B. Kostkin, G. V. Makukhina, L. I. Khozhai, and V. A. Otellin, "A conditioned passive avoidance reflex reaction in female and male rats subjected to hypoxia at different stages of prenatal development," *Zh. Évolyuts. Biokhim. Fiziol.*, **40**, No. 3, 250–253 (2004).
3. N. M. Dubrovskaya, N. N. Nalivaeva, A. J. Turner, and I. A. Zhuravin, "Effects of an α-secretase inhibitor metabolizing the amyloid peptide precursor on the formation of memories in rats," *Zh. Vyssh. Nerv. Deyat.*, **55**, No. 6, 725–728 (2005).
4. I. A. Zhuravin, "Formation of integral mechanisms of regulation of motor functions in mammals depending on the conditions of embryonic development," *Zh. Évolyuts. Biokhim. Fiziol.*, **38**, No. 5, 478–484 (2002).
5. I. A. Zhuravin and N. M. Dubrovskaya, "Involvement of the cholinergic system of the sensorimotor cortex of the rat brain in regulating different types of movement," *Zh. Vyssh. Nerv. Deyat.*, **50**, No. 1, 103–112 (2000).
6. I. A. Zhuravin, N. M. Dubrovskaya, and N. L. Tumanova, "Postnatal physiological development of rats after acute prenatal hypoxia," *Ros. Fiziol. Zh.* **89**, No. 5, 522–532 (2003).
7. I. A. Zhuravin, N. N. Nalivaeva, and N. M. Dubrovskaya, "Effects of exogenous gangliosides on the formation of operant movements with tactile control in rats," *Zh. Vyssh. Nerv. Deyat.*, **43**, No. 3, 1129–1136 (1993).
8. V. G. Kassil', V. A. Otellin, L. I. Khozhai, and V. B. Kostkin, "Critical periods in the development of the brain," *Ros. Fiziol. Zh.* **86**, No. 11, 1418–1425 (2000).
9. K. Yu. Reznikov, *Proliferation of Brain Cells in Vertebrates in Conditions of Normal Brain Development and Trauma* [in Russian], Nauka, Moscow (1981).
10. S. S. Trofimov, R. U. Ostrovskaya, N. M. Smol'nikova, E. P. Nemova, T. A. Gudasheva, E. A. Kuznetsova, and T. A. Voronina, "Correction of cognitive impairments induced by intrauterine hypoxia by nooglutil and L-pyroglutamyl-D-alanine in rats," *Eksperim. Klin. Farmakol.*, **58**, No. 6, 10–13 (1995).
11. J. Altman and K. Sudarshan, "Postnatal development of locomotion in the laboratory rat," *Anim. Behav.*, **23**, 896–920 (1975).
12. K. K. Ashton, J. Zapf, I. Einschenk, and I. Z. MacKenzie, "Insulin-like growth factors (IGF) 1 and 2 in human foetal plasma and relationship to gestational age and foetal size during midpregnancy," *Acta Endocrinol.*, **110**, No. 4, 558–563 (1985).
13. R. E. Burke and K. G. Baimbridge, "Relative loss of the striatal striosome compartment, defined by calbindin-D28k immunostaining, following developmental hypoxic-ischemic injury," *Neurosci.*, **56**, No. 2, 305–315 (1993).
14. M. Dubovicky, E. Ujhazy, P. Kovacovsky, J. Navarova, M. Jurani, and L. Soltes, "Effect of melatonin on neurobehavioral dysfunctions induced by intrauterine hypoxia in rats," *Cent. Eur. J. Public Health*, **12**, 23–25 (2004).
15. H. Golan and M. Huleihel, "The effect of prenatal hypoxia on brain development: short- and long-term consequences demonstrated in rodent models," *Dev. Sci.*, **9**, No. 4, 338–349 (2006).
16. R. H. Hermans, R. F. McGivern, W. Chen, and L. D. Longo, "Altered adult sexual behavior in the male rat following chronic prenatal hypoxia," *Neurotoxicol. Teratol.*, **15**, No. 6, 353–363 (1993).
17. B. Janicke and H. Coper, "The effect of prenatal exposure to hypoxia on the behaviour rats during their life span," *Pharmacol. Biochem. Behav.*, **48**, 863–873 (1994).
18. P. Lipton, "Ischemic cell death in brain neurons," *Physiol. Rev.*, **79**, 1431–1568 (1999).
19. M. W. Miller, "Effects of prenatal exposure to ethanol on neocortical development: II. Cell proliferation in the ventricular and subventricular zones of the rat," *J. Comp. Neurol.*, **287**, 326–338 (1989).
20. G. D. Muir, "Early ontogeny of locomotor behaviour: a comparison between altrical and precocial animals," *Brain Res. Bull.*, **53**, No. 5, 719–726 (2000).
21. N. N. Nalivaeva, L. Fisk, R. M. Canet Aviles, S. A. Plesneva, I. A. Zhuravin, and A. J. Turner, "Effect of prenatal hypoxia on expression of amyloid precursor protein and metallopeptidases in the rat brain," *Lett. Peptide Sci.*, **10**, 455–462 (2004).
22. C. Nyakas, B. Buwalda, and P. D. M. Luiten, "Hypoxia and brain development," *Prog. Neurobiol.*, **49**, No. 1, 1–51 (1996).
23. G. Pepeu and M. G. Giovannini, "Changes in acetylcholine extracellular levels during cognitive processes," *Learn. Mem.*, **11**, No. 1, 21–27 (2004).
24. J. A. Prada and R. C. Tsang, "Biological mechanisms of environmentally induced causes of IUGR," *Eur. J. Clin. Nutr.*, **52**, S21–S28 (1998).
25. Y. Qi and Q. M. Xue, "Ganglioside levels in hypoxic brains from neonatal and premature infants," *Mol. Chem. Neuropathol.*, **14**, No. 2, 87–97 (1991).
26. H. Rahman, H. Rosner, and K. H. Kortje, "Ca^{2+}-Ganglioside interaction in neuronal differentiation and development," in: *Biological Function of Gangliosides*, L. Svenerholm (ed.), Elsevier, Amsterdam (1994), Vol. 101, pp. 127–145.
27. S. Rees and T. Inder, "Fetal and neonatal origins of altered brain development," *Early Hum. Dev.*, **81**, No. 9, 753–761 (2005).
28. H. F. Sadiq, U. G. Das, T. F. Tracy, and S. U. Devaskar, "Intrauterine growth restriction differentially regulates perinatal brain and skeletal muscle glucose transporters," *Brain Res.*, **823**, 96–103 (1999).
29. K. V. Sharma, C. Koenigsberger, S. Brimijoin, and J. W. Bigbee, "Direct evidence for an adhesive function in the noncholinergic role of acetylcholinesterase in neurite outgrowth," *J. Neurosci. Res.*, **64**, 165–175 (2001).
30. F. Thullier, R. Lalonde, X. Cousin, and F. Lestienne, "Neurobehavioral evaluation of lusher mutant mice during ontogeny," *Dev. Brain Res.*, **100**, 22–28 (1997).