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Prenatal Hypoxia Impairs Olfactory Function in Postnatal Ontogeny in Rats

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Analysis of the age-related dynamics of olfactory behavior in the odor preference and food search tests showed that all male Wistar rats, regardless of age, preferred valerian essential oil, whose components have the properties of pheromones in rodents, when given a selection of eight essential oils; young rats displayed better food-seeking results than adult and old animals. Acute prenatal hypoxia (PH) on E14 (7% O_2 for 3 h) led to impairment of the valerian odor preference at all ages studied and to decreased productivity of food searches. Neurodegenerative processes were seen in the piriform cortex after PH, with reductions in the number of neurons and increases in glial elements. We have previously observed these changes in the entorhinal cortex and hippocampus, but not in the olfactory bulbs. This suggests that PH-induced decreases in olfactory function in rats may result from impairments to the formation of the central elements of the analyzer during the first months of postnatal ontogeny.

Keywords: postnatal ontogeny, prenatal hypoxia, neurodegeneration, olfaction, piriform cortex, olfactory bulb, odor preference, food search.

The article is dedicated to the memory of Doctor of Biological Sciences Igor Aleksandrovich Zhuravin

Introduction. Olfactory function is one of the main sensory modalities in animals enabling their survival and accurate orientation in the environment and supporting various types of social communication. Particular odors can alter behavioral reactions in animals [Belyakov et al., 2015]. Different odor stimuli are perceived by a large number of olfactory receptors in one of the largest multigene families in mammals [Niimura, 2009]. Olfactory signal perception starts in the olfactory epithelium, which contains olfactory receptors, from which signals pass to the olfactory bulb and thence to the cortical areas of the brain for analysis and memorization [Mori and Sakano, 2011; Suzuki and Osumi, 2015; Takahashi et al., 2018].

The development of the olfactory system in animals and humans starts during the embryonic period and the system is quite well formed by birth, providing the neonatal body with rapid orientation outside the mother's body [Sarnat and Fores-Sarnat, 2019]. Olfactory sensitivity and odor perception are subject to ontogenetic dynamics [Apfelbach et al., 1991] and can change depending on the animal's physiological state [Brennan and Kendrick, 2006] and also depending on the surrounding olfactory environment or the prior history of olfactory contacts [Sokolov and Voznesenskaya, 1996]. Impaired olfaction leads to significant changes in animals' behavior. Thus, removal of the olfactory bulbs affects both the animals' motor reactions, inducing hyperlocomotion, and their psychoemotional state [Nedogreeva et al., 2020]. Studies of bulbectomized animals have identified a wide spectrum of morphological and biochemical changes in the brain, which are also seen on development of neurodegenerative diseases in humans (for review see [Gulyaeva et al., 2017].

Previous studies in our laboratory showed that prenatal hypoxia (PH) on day 14 of embryonic development in rats led to impairments to the formation and morphofunctional properties of the parietal and entorhinal cortex, as well as the hippocampus, which during postnatal ontogeny were accompanied by decreases in dendritic spine density and

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delayed neurogenesis and late formation of nervous tissue plasticity [Vasile'ev et al., 2020; Tumanova et al., 2021; Vasilev et al., 2016]. PH also significantly alters the molecular characteristics of nervous tissue, leading to impairments to the cholinergic [Morozova et al., 2020] and peptidergic systems. In particular, rats subjected to PH showed decreased expression and activity of the neuropeptidase neurolysin (NEP) [Nalivaeva et al., 2012; Vasilev et al., 2021], which cleaves one of the mediators of olfactory transmission – somatostatin [Barnes et al., 1995; Nocera et al., 2019]. At the behavioral level, PH leads to impaired cognitive functions in rats during postnatal ontogeny [Dubrovskaya and Zhuravin, 2009], though no studies addressing the effects of PH on olfactory behavior in rats during postnatal development and on the formation of the structures supporting the conduction of olfactory stimuli have been performed.

The aim of the present work was to investigate the olfactory behavior of rats (odor preference and odor-based food seeking) at different stages of ontogeny (the prepubertal period, adulthood, old age) during normal postnatal development and after acute PH on day 14 of pregnancy. The structures of the rat piriform cortex were also analyzed, these being an important component in their olfactory analyzer.

Methods. ***Animals.*** Experiments used Wistar rats from the supplier Rappolovo. Animals were kept in in standard animal house conditions at the Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, with a 12 h day/12 h night light regime in groups of 4–5 individuals per cage with free access to water and food, except as stated in the text. Experiments were run in compliance with the protocol for use of laboratory animals of the Sechenov Institute of Evolutionary Physiology and Biochemistry, which is based on the European Communities Directive #86/609 for the Humane Care of Experimental Animals.

Model of prenatal acute normobaric hypoxia. Offspring meeting the experimental requirements were obtained by placing virgin females aged 3–4 months and weighing 250–260 g with males in pairs in cages in the animal house. The first day of pregnancy was taken as the day on which spermatozoids were seen in vaginal smears. Fertilized females were placed in animal-house cages in groups of 4–5 animals. on day 14 of pregnancy (E14), females of the experimental group (prenatal hypoxia – PH) were subjected to normobaric hypoxia in a special chamber (7% O_2, 3 h), as described previously [Dubrovskaya and Zhuravin, 2009]. Pregnant females of the control group (C) were also placed in the chamber, but with a normal oxygen content. On day 20 of pregnancy, females of the experimental and control groups were placed in separate cages. Taking day 0 as the day the animals were born, each litter was culled to eight individuals on day 2 after birth (P2). Rat pups were separated from their mothers on P25, separated by sex, and placed in cages containing 4–5 individuals per cage in standard conditions; one cage could contain males from different litters. No mortality was seen among the animals during the experiments. Rats (males) of both groups (C and PH) were investigated at different stages of ontogeny at ages 1 month (the prepubertal period; "young"), three months (adulthood, "adults"), and 1.5 years (aged rats, "old"). The numbers of animals in each group are indicated in the figure legends.

Odor preference test. in the odor preference paradigm, animals were simultaneously presented with samples in eight open glass bowls (Ø = 30, h = 30 mm) containing essential oils of orange, clove, jasmine, mint, eucalyptus, wormwood, lavender, and valerian, in volumes of 0.1 ml, once in eight days. Bowls were positioned in a circle at equal distances from each other and from the wall of the special chamber of size 500 × 500 × 200 mm (for young rats) or 1000 × 1000 × 400 mm (for adult and old rats). Animals were placed in the center of the chamber and the numbers of approaches/sniffs in each bowl were recorded. For each new presentation, the positions of the bowls with odor samples were changed. After each animal, the chamber floor was wiped with 50% ethanol. The attractiveness of each odor was expressed as a percentage of the total number of sniffs averaged for all test days. Of the whole set of odors, only valerian has a functionally significant pheromone effect for rodents [Mel'nik et al., 2009].

Food search productivity test. The food search paradigm used our modification of the food search method [Sun et al., 2016]. Animals were tested in a special chamber of area 100 × 100 cm with 16 apertures of diameter 2 cm and opaque walls 30 cm in height. During the experiment, two of the apertures in the floor were covered, each by one piece of oatmeal cookie 0.5 cm in diameter. Pieces of cookie were placed beneath the litter layer 0.5 cm below the surface of the chamber floor and their distribution was altered for each new test. Before testing, rats were deprived of food for two days. During the daily 15-min tests (once in six days), the numbers of pieces of food grasped were counted (0, 1, or 2) and the time taken to find them was recorded. In addition, one test cycle was taken as the total number of sniffs of all openings in the floor of the experimental chamber. The test was stopped after the animal found the second piece of cookie. Prior to each presentation, the floor of the chamber was wiped with 50% ethanol solution and the upper layer of litter was changed.

Light microscopy. Light optical studies were performed on rats aged P20, one month (P35), and three months (P90) in the control group ($n = 8$ in each age group) and the experimental group ($n = 9$ in each age group). After transcardiac perfusion with paraformaldehyde solution (4% in 0.1 M phosphate buffer pH 7.4), brains were fixed in the same solution for four days and then placed in 20% sucrose solution for one day. Frozen frontal brain sections of thickness 20 μm were cut on a Leica CM 1510S cryostat (Leica Microsystems, Germany). Studies were performed on selected sections of the piriform cortex of the brain (2.3–4.5 mm from the bregma) [Paxinos and Watson, 2007], which were stained by the Nissl method. An ImagerA mi-

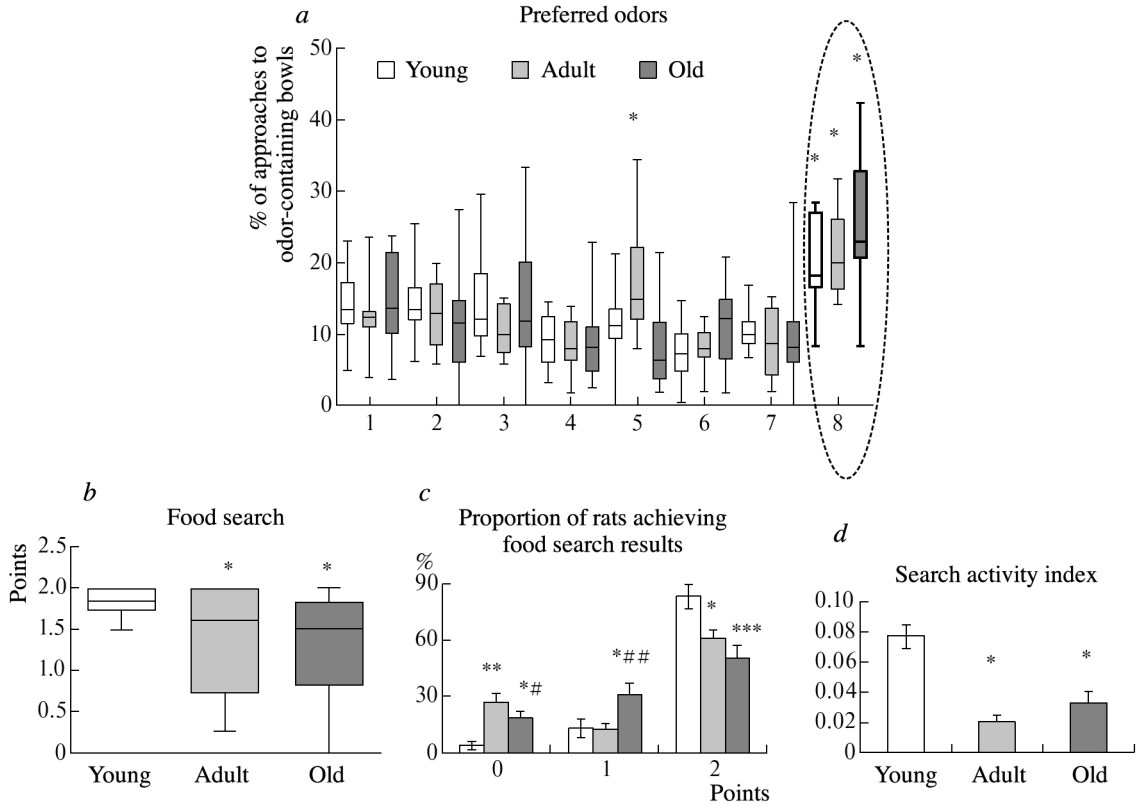

Fig. 1. Age-related dynamics of olfactory behavior in rats. *a*) Behavior in the odor preference test in young ($n = 16$), adult ($n = 13$), and old ($n = 13$) rats in the control group. The ordinate shows the number of approaches too each odor-containing bowl, % of total number of approaches to all bowls, which was taken as 100%. The abscissa shows odors: *1*) orange; *2*) clove; *3*) jasmine; *4*) mint; *5*) eucalyptus; *6*) wormwood; *7*) lavender; *8*) valerian. *$p < 0.001$ – differences between the odors of valerian and other oils in each age group. *b–d*) Behavior in the food search test in young ($n = 15$), adult ($n = 13$), and old ($n = 14$) rats of the control group. *b*) Mean food search results, points(ordinate) for each age group (abscissa), *$p < 0.05$ – between-group differences; *c*) proportion of rats, % of total number of rats in each group (ordinate) achieving results from 0 to 2 (abscissa). *a–b*) Data presented as median and interquartile ranges showing spread between the minimum and maximum values. *c–d*) Data presented as mean ± error of the mean. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ – differences between young and adult or young and old rats; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ – differences between adult and old rats; *d*) Search activity index (ordinate). *$p < 0.001$ – differences between young and adult or young and old rats.

croscope (Zeiss, Germany) was used to evaluate the state of nervous tissue on series of sections of thickness 20 μm, selecting the first section in the series at random. The distance between sequential sections in the series was 40 μm. Analyses were run in the VideoTest Master-Morphology (Video-Test, St. Petersburg, Russia). Six sections from each animal were used to compute the mean total number of cells in pieces of tissue of area 10000 μm^2, as described in our previous reports [Vasilev et al., 2008]. Counting included only those cells in which the section passed through the cell nucleus. The set of pyramidal neurons included cells with extended bodies, profile field areas of >25 μm^2, and a cell body long axis:short axis ratio of >2. The set of non-pyramidal neurons included round cells with body areas of >25 μm^2 and a cell body long axis:short axis ratio of <2. Cells with areas of stained profile field areas of <25 μm^2 were counted as glial cells.

Statistical analysis. Statistical data processing was run in SPSS SigmaStat 3.0 or GraphPad Prism 98, using one-way ANOVA followed by the post hoc Fisher LSD test for behavioral experiments or the Mann–Whitney U test for morphological experiments. Analysis of values with normal distributions used parametrical statistical methods. Differences were regarded as significant at $p \le 0.05$. All data are presented as mean ± error of the mean except as otherwise stated.

Results. ***Studies of odor preference in the postnatal ontogeny of control rats.*** Comparative analysis of the behavior of young, adult, and old rats in the odor preference test showed that all intact rats, regardless of age, were most attracted by valerian oil (Fig. 1, *a*), the number of approaches to which dominated over others: 19.9 ± 2.0% in young animals ($F_{7,127} = 11.1$, $p < 0.001$), 20.0 ± 0.04% in adult animals ($F_{7,103} = 11.8$, $p < 0.001$), and 25.5 ± 0.02% ($F_{7,103} = 8.3$, $p < 0.001$). In adult rats, particular preference was also enjoyed by the odors of eucalyptus, with a mean 16.5 ± ± 0.02% of approaches to the bowl containing this odor ($F_{7,103} = 11.8$, $p < 0.05$). There were no statistically significant differences in the numbers of expressions of preference for the odor of valerian in animals of different ages.

Changes in food search productivity in postnatal ontogeny in rats. The control group of animals showed age-re-

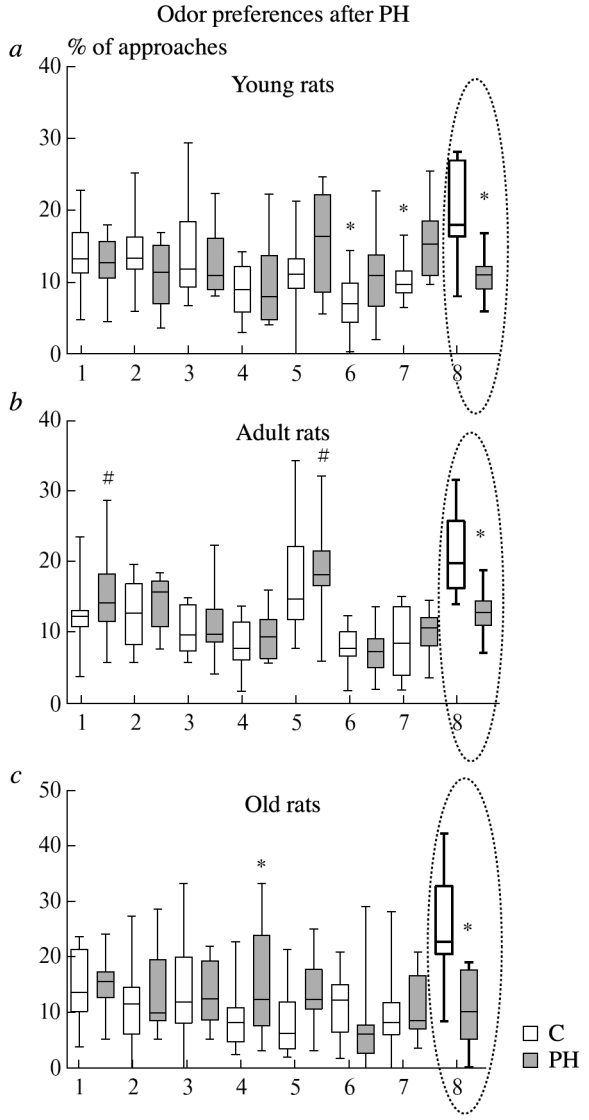

Fig. 2. Comparative analysis of odor preference at different stages of ontogeny between control and PH rats. *a*) Comparison of young control ($n = 16$) and PH ($n = 10$) animals; *b*) comparison of adult control ($n = 13$) and PH ($n = 14$) animals; *c*) comparison of old control ($n = 13$) and PH ($n = 11$) animals. For further details see caption to Fig. 1. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ – differences between the odor of valerian and other odors in adult PH animals. $^{*}p < 0.05$, $^{**}p < 0.001$ – differences between control and PH groups.

lated changes in food search productivity ($F_{2,41} = 4.5$, $p = 0.017$) (Fig. 1, *b*). Comparison of mean food search productivity scores in different age groups showed that young rats demonstrated better results than adult ($p = 0.013$) and old ($p = 0.014$) animals. The young animals group was dominated by individuals searching for food with 100% productivity ($F_{2,16} = 9.9$, $p < 0.01$) and there were fewer animals with a zero search results ($p < 0.05$) (Fig. 1, *c*). Adult and old animals showed no differences in terms of mean productivity scores (Fig. 1, *b*), though among old rats there were about twice as many ($F_{2,16} = 7.6$, $p < 0.01$) individuals carrying out searches with 50% results as compared with young and adult rats (Fig. 1, *c*), while among adult rats there were more individuals carrying out unproductive food searches ($F_{2,16} = 11.4$, $p < 0.05$) (Fig. 1, *c*).

Search activity was evaluated by assessing the ratio of the total number of sniffs of all openings to test duration in seconds and this was taken as an index reflecting the food search motivation of the animals. In young control rats, the search activity index was 3.7 and 2.3 times greater than that in adults and old animals, respectively, ($F_{2,41} = 22.2$, $p < 0.01$) (Fig. 1, *d*).

Effects of PH on odor preference in postnatal ontogeny in rats. PH rats showed significant changes in behavior in the odor preference test. Thus, young (Fig. 2, *a*) and old (Fig. 2, *c*) PH rats did not display any preference for any of the odors presented, while adult rats showed a preference ($F_{7,111} = 11.0$, $p < 0.001$) for orange and eucalyptus odors over other odors (Fig. 2, *b*). Preference for valerian odor in PH rats was 45% lower in young ($F_{15,203} = 5.6$, $p < 0.001$), 23% lower in adult ($F_{15,215} = 10.8$, $p < 0.001$) in adults, and 38% lower in old rats ($F_{15,191} = 4.5$, $p < 0.001$) as compared with controls. Also in comparison with controls, young PH animals were more occupied with the odors of wormwood and lavender ($F_{15,203} = 5.6$, $p < 0.001$), while for old animals the most attractive odor was that of mint ($F_{15,191} = 4.5$, $p < 0.033$). The impairment to preference for valerian odor among other indifferent odorants was interpreted as evidence of impairment to the normal operation of the olfactory analyzer.

Effects of prenatal hypoxia on food search productivity in postnatal ontogeny in rats. Analysis of the behavior of PH animals revealed the absence of any age-related changes in food search productivity ($F_{2,32} = 0.3$, $p = 0.758$). On average, PH rats of different age groups had lower scores than their control peers ($F_{5,74} = 7.2$, $p < 0.001$). Young animals showed a 43% decrease in this measure ($p < 0.01$), while adults showed a 40% decrease ($p = 0.018$) and old animals a 41% decrease ($p < 0.01$) (Fig. 3, *a*). More detailed analysis showed that the PH group displayed no differences in the numbers of rats carrying out unsuccessful or 100% searches (Fig. 3, *b*, *d*). However, age-related changes were seen in the numbers of rats finding only one cookie piece ($F_{2,16} = 3.7$, $p = 0.05$), and the number of young rats with this result was smaller than that of old animals ($p < 0.01$) (Fig. 3, *c*). At all the stages of ontogeny studied, comparison with control peers showed that PH rats had a higher frequency ($F_{5,33} = 11.7$, $p < 0.001$) of unsuccessful searches with zero results (Fig. 3, *b*) and, more rarely ($F_{5,33} = 10.5$, $p < 0.001$) found both cookie pieces. (Fig. 3, *d*).

In terms of the search activity index, PH animals were no different from control peers and showed similar ontogenetic dynamics in this indicator ($F_{5,74} = 16.9$, $p < 0.01$). In young PH rats, this indicator was 3.1 and 3.7 times higher than in adult and old rats, respectively, (Fig. 3, *e*).

Analysis of the structure of the rat piriform cortex during postnatal ontogeny. Nissl light microscopic investi-

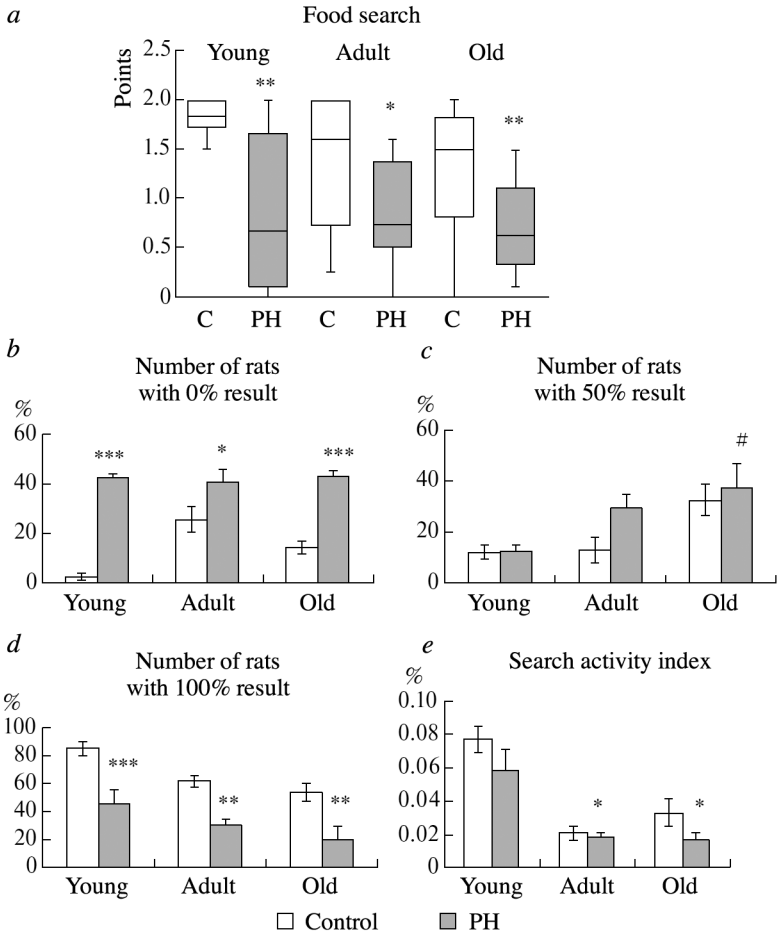

Fig. 3. Comparative analysis of food search effectiveness in control and PH rats. *a*) The ordinate shows group mean search productivity in points. For further details see caption to Fig. 1. $^{*}p < 0.05$, $^{***}p < 0.001$ – differences between control and PH groups. *b–d*) The ordinate shows the proportion of rats (%) achieving results in the food search among the total number of rats in each age group, which was taken as 100%. *b*) Young control ($n = 16$) and PH ($n = 10$) rats; *c*) adult control ($n = 13$) and PH ($n = 14$) rats; *d*) old control ($n = 13$) and PH ($n = 11$) rats. Data are presented as mean ± error of the mean. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ – differences between young and old PH rats; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ – differences between control and PH groups. *e*) The ordinate shows the search activity index. $^{*}p < 0.001$.

gations of the cellular organization of the rat piriform cortex, which is the center to which the olfactory pathways from the olfactory bulbs run, showed that at P20, rats after PH, as compared with controls, had signs of destruction of nerve cells, in particular swollen neurons with edematous processes. The cytoplasm of these neurons contained vacuoles and organoid lysis (chromatolysis) (Fig. 4, *e*). At this stage, there was also a large number of glial cells located close to swollen neurons. At P35, the number of glial cells increased and the number of neurons decreased (Fig. 4, *i*). At P90, the piriform cortex of PH rat pups showed swelling of neurodegenerative processes and groupings of glial cells disappeared, though occasional neurons in the state of chromatolysis were still found (Fig. 4, *d*, *g*).

Morphometric studies of the cellular composition of rat piriform cortex tissue after PH. Analysis of the cellular composition of the piriform cortex of PH rats on P35 demonstrated a 19.3% decrease in the total number of cells as compared with controls (Mann–Whitney test, U = 12.4, $p = 0.03$) (Fig. 4, *h*, *i*). PH rats also showed a decrease in the number of pyramidal neurons, by 25.2% of the control level (Mann–Whitney test, U = 8.2, $p < 0.01$), with no statistical differences in the numbers of nonpyramidal neurons (Mann–Whitney test, U = 21.0, $p < 0.08$). This is evidence of the death of projection pyramidal neurons in the piriform cortex of PH rats during the first month of development. At the same time, the mean number of glial cells in these animals was on average 20.4% greater than that in controls (Fig. 4, *b*), though the inhomogeneity of the distribution of these cells had the result that these differences did not reach statistical significance (Fig. 4, *e*, *f*).

Discussion. Decreased olfactory function in animals and humans is seen both in normal aging and at earlier ages on development of viral infections, which is particularly apparent in the COVID-19 pandemic [Koralnik and Tayler, 2020], and also in neurodegenerative diseases such as

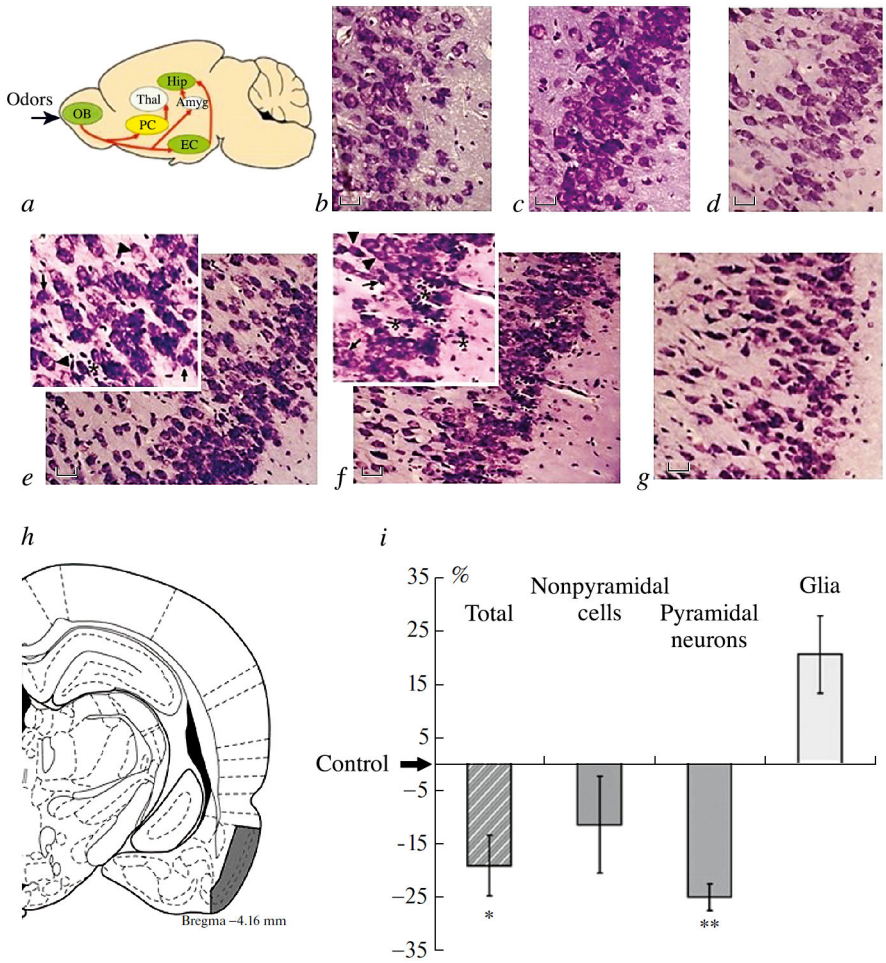

Fig. 4. Structural changes in the piriform cortex in postnatal ontogeny in normal conditions and after PH. *a*) Diagram showing connections in the rat olfactory analyzer. OB – olfactory bulb; Thal – thalamus; Hip – hippocampus; EC – entorhinal cortex; PC – piriform cortex; *b–g*) Microphotographs of Nissl-stained areas of the piriform cortex in rats; scale bars 30 μm. *b–d*) Microphotographs of areas of the piriform cortex of control rats on P20 (*b*), P35 (*c*), and P90 (*d*). *e–g*) Microphotographs of areas of the piriform cortex in PH rats on P20 (*e*), P35 (*f*) and P90 (*g*). The upper left parts of (*e*) and (*f*) show enlarged fragments of microphotographs with groupings of glial elements and degenerating neurons. Thin black arrows show nonpyramidal neurons; black triangles show pyramidal neurons; *groupings of glial cells. *h*) Diagram showing the location of the study area of the piriform cortex used for morphometric analysis. *i*) Changes in the numbers of different cell populations (abscissa) in the area of the rat piriform cortex studied on P35. The ordinate shows results of changes after PH, % of the control level taken as 0%, expressed as mean ± error of the mean. *$p < 0.05$, **$p < 0.01$.

Alzheimer's disease (AD) [Murphy, 2019], Parkinson's disease [Doty, 2012], and other nervous system pathologies [Barresi et al., 2012; Bsteh et al., 2019; Carenomolla et al., 2020]. Olfactory disorders in the pathogenesis of neurodegenerative diseases appears much earlier than cognitive or motor impairments [Bsteh et al., 2019]. One view is that they are based on molecular and cellular mechanisms different from those of normal aging [Parvand and Rankin, 2020], which makes investigations in animal models an important direction in this area of neurobiology.

As formation of the olfactory system starts in the embryonic period, harmful prenatal actions on the body can lead to significant rearrangements in the development of the neural networks involved in conducting olfactory stimuli [Akers et al., 2011; Liu et al., 2013] and impairments of olfactory function in subsequent ontogeny [Franks and Isaacson, 2005]. Our studies showed that prenatal hypoxia significantly alters olfactory behavior in rats. The methods used for testing rat olfactory behavior in this study were based both on their ability to recognize physiologically significant odorants and on targeted odor-based food searches. Among the eight natural essential oil odors, the most attractive for all intact rats, regardless of age, has been shown to be valerian oil. This result is entirely consistent in view of the fact that the components of valerian oil, particularly isovaleric acid, have pheromone value for this animal species and is widely used in studies of olfactory function in rodents [Boryakova et al., 2007; Mel'nik et al., 2009; Mel'nik et al., 2012]. PH had significant influence on odor preference in all age groups. It is of note that after PH, young and old rats showed no preference for any of the odors presented, and only adult animals displayed preference for the odors of

orange and eucalyptus. Thus, PH eliminated the preference for the odor of valerian, which is physiologically significant for normal animals; this can have pernicious influences on their sexual behavior.

Analysis of the rats' olfactory behavior in terms of seeking food, the productivity of which can be influenced by both the animal's motivation and the state of its olfactory system [Roesch et al., 2007; Bianchi et al., 2014], showed that the effectiveness of the food search in control rats, like the search activity index, decreased with age. It can therefore be suggested that decreased search motivation in adult and old rats results in the lower productivity. PH did not alter the age-related dynamics of the search activity index in rats, though food search productivity in PH animals was significantly lower than that in intact peers and search results in these animals were often zero and rarely 100%. These results suggest the conclusion that PH impairs olfactory function in the animals, not the motivation for seeking food. This is consistent with numerous published data showing that PH and other pathological actions impair more complex forms of olfactory behavior linked with associative learning and memory [Tyul'kova et al., 2010; Akers et al., 2011].

The operation of the olfactory analyzer involves many parts of the brain, though the most important are the olfactory bulb, which is responsible for the initial processing of signals arriving from olfactory epithelium receptor cells in the nasal cavity and vomeronasal organ, and the piriform and entorhinal areas of the cortex, which contain projection neurons sending signals to the thalamic nuclei and hippocampus, as well as the hypothalamic nuclei and amygdala, which modulate the incoming information [Arzi and Sobel, 2011; Ghosh et al., 2011; Sosulski et al., 2021] (Fig. 4, *a*). Unfortunately, information on the influences of prenatal hypoxia on these parts of the brain is very scanty in the literature. We have previously shown that PH at E14 impairs neuroblast generation and migration on formation of the entorhinal areas of the rat cerebral cortex, and also produces neuron death in the first month of postnatal ontogeny [Vasilev et al., 2020; Tumanova et al., 2021]. The present studies using the same model showed that PH leads to neuron death in the piriform area of the rat cerebral cortex. This is evidence supporting the existence of common molecular-cellular mechanisms of impairments tot formation of the cortical plate in different parts of the cerebral cortex in rodents during embryonic development. In all cases, changes in the structural organization of the cortex after PH were most marked in the second half of the first month of postnatal development in rats (P20–30). At this time, they also showed the most severe motor and cognitive dysfunctions [Dubrovskaya and Zhuravin, 2009] and impairments to odor-based food seeking and changes in preferences for odorants, as demonstrated in this study. Nonetheless, while the nature of structural changes in the cortical plate were very similar, the patterns of changes in cell composition in various parts of the cortex after PH were different. Thus, the elements of the parietal cortex most sensitive to PH were projection pyramidal neurons, especially in layers V–VI, with no marked gliosis [Vasilev et al., 2016], while notable reductions in the numbers of nonpyramidal cells were seen in the entorhinal cortex [Vasilev et al., 2020; Tumanova et al., 2021]. According to this study, PH also led to decreases in the numbers of projection pyramidal neurons in the piriform area of the cortex, though this was characterized by the presence of local groupings of glial cells [Tumanova et al., 2021].

Our previous results also provide evidence that PH induces pathological changes not only in the piriform and entorhinal cortex, but also in the hippocampus [Tumanova et al., 2021], i.e., in those areas of the brain involved in processing olfactory information. At the same time, PH did not induce any significant changes in the composition of the olfactory bulbs, which is characterized by a high level of neurogenesis during the whole of postnatal development [Bianchi et al., 2014]. It can be suggested that projection neuron death in the cortical areas is also among the causes of impaired odor-based food seeking and discrimination of odorants, as seen during the present study.

The neuropeptidase neprilysin (NEP) is another important element in the conduction of olfactory stimuli; this enzyme cleaves somatostatin, which is an important mediator of peptidergic transmission in different parts of the brain, including the olfactory bulbs [Barnes et al., 1995; Nocera et al., 2019]. NEP in the brains of developing rat embryos starts to be expressed before birth in the structures of the olfactory bulbs [Dutriez et al., 1992]. As demonstrated by our previous studies, the expression level of NEP in this part of the olfactory analyzer in rats is greater than that in the cortical structures of the brain throughout the postnatal period and decreases only in old rats, correlating with decreases in the acuity of their olfactory function [Vasilev et al., 2021]. PH on E4 significantly decreased the level of NEP expression in the entorhinal and parietal cortex, as well as in the hippocampus of rats in the first month after birth, though it had no effect on the level of NEP expression in the olfactory bulbs [Vasilev et al., 2021]. These data support our hypothesis that PH mainly alters the properties of the central components of the olfactory analyzer. As NEP is one of the main amyloid-degrading enzymes in brain tissue, deficit in its content and activity play an important role in the pathogenesis of AD (for review see [Nalivaeva and Turner, 2019]); decreases in its expression in the olfactory structures of the brains of individuals exposed to prenatal stress and during normal aging may promote β-amyloid peptide accumulation within them, nerve cell death, and neurodegeneration, increasing the risk of developing AD and promoting decreases in olfactory function [Saiz-Sanchez et al., 2010].

Conclusions. During the postnatal development of rats, there is a reduction in the effectiveness of olfactory function, apparent as lower levels of food search productivity. Hypoxia in the prenatal period leads to decreases in the ef-

fectiveness of seeking food, starting from the early stages of ontogeny and continuing to old age, with impairment to recognition of the odor of valerian, which has pheromonal importance, at all stages studied. PH-induced impairments correlate with changes in the structure and cell composition of the piriform cortex, which is an important component part of the olfactory analyzer, as well as the entorhinal cortex and hippocampus, but not the olfactory bulbs. This leads to the conclusion that PH during the period of active formation of cortical brain structures in rats significantly impairs the processes underlying the development and biochemical characteristics of the central parts of the olfactory analyzer, without significantly affecting its peripheral components, leading to impaired realization of the animal's olfactory behavior.

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