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Dedicated to the 90th anniversary of Margarita Gennadievna Belekhova, a wonderful person, researcher, and evolutionary biologist

A Study of Neurodegenerative Changes in the CA1 Region of the Dorsal Hippocampus in Adult Rats with Prenatal Hyperhomocysteinemia

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Abstract—The work is devoted to the study of neurodegenerative changes in the ultrastructural organization of hippocampal CA1 in adult rats that have suffered prenatal hyperhomocysteinemia (pHHC). Electron microscopy in the neural networks of the CA1 region of the dorsal hippocampus in adult rats with pHHC, in contrast to control animals, revealed signs of pathological changes: degeneration of pyramidal neurons and destruction of the myelin sheath of axons, as well as destruction of the axial cylinders of basal and apical dendrites directed from a pyramidal layer of neurons in the direction of *tractus temporammonic* or Schaffer collaterals, respectively. In control animals, on the distal branches of dendrites in the layers of the *stratum oriens* and *stratum radiatum*, using the Golgi method, a dense network of varicose dendritic extensions was identified, providing an increase in the area of synaptic contacts. In rats that have undergone pHHC, significant destructive changes are found in these dendritic varicosities: destruction of mitochondrial cristae and the appearance of dilated cisterns. In adult rats with pHHC, it completely eliminates the preference for the smell of valerian, which is normally a physiologically significant stimulus, which indicates a negative effect of pHHC on the functioning of the olfactory analyzer, the activity of which is closely connected with the hippocampus. The obtained facts indicate the detrimental effect of homocysteine on the structure and interneuronal connections in the nervous tissue of the CA1 region of the dorsal hippocampus, as a morphological substrate for the integration of stimuli entering it.

Keywords: rat, hippocampus, ontogeny, hyperhomocysteinemia, ultrastructural organization, varicose dendrites, neurodegenerative changes, sense of smell

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Hyperhomocysteinemia (HHC) is a condition of the body with an increased level of toxic amino acid homocysteine in tissues, which can cause excitotoxic death of brain neurons and dysfunction of various body systems. In the case of HHC, the mother's body experiences a disturbance in placental blood flow and the production of a number of trophic factors, which, in turn, can lead to serious changes in the process of formation of the offspring's brain during embryogenesis, as well as its maturation and functioning in early postnatal ontogenesis (Vasilev et al., 2023). In the cortex and hippocampus of the brain of the offspring of rats exposed to experimentally induced prenatal HHC (pHHC), in the period from the fourth day of pregnancy to delivery, we previously identified signs of developmental delay and neurodegenerative changes (Shcherbitskaia et al., 2021). Among the most characteristic structural changes, the death of projection pyramidal neurons in the neocortex and dorsal hippocampus was noted against the background of the development of the process of neuroinflammation. Neuroinflammatory processes reached their greatest severity in the CA1 region of the dorsal hippocampus, which is an important integrative center closely connected with the neocortex. The CA1 region plays an important role in behavioral responses to internal and external olfactory, somatosensory, auditory, and visual information, and also takes an active part in organizing complex forms of behavior in rats. The development of the neurodegenerative process caused by pHHC was accompanied by a dysfunction of synaptic

Abbreviations: HHC—hyperhomocysteinemia; pHHC—prenatal HHC; GO—oligodendrocyte glycoprotein; ANOVA—analysis of variance.

transmission, which, in particular, was expressed in a decrease in long-term potentiation and a decrease in the pool of labile mushroom-shaped dendritic spines with a spiny apparatus involved in its provision (Postnikova et al., 2022), which led to a cognitive deficit.

The olfactory system in animals is critical for survival during tasks such as choosing food and identifying the scent of a predator. It also has a significant impact on social interactions, reproduction, and many other behaviors. Olfaction is unique among sensory systems in its relative structural conservation throughout mammalian evolution. Compared with other sensory systems, the primary olfactory cortex, including the anterior olfactory nucleus, olfactory tubercle, and piriform cortex, has been shown to have functional connectivity with hippocampal neural networks (Zhou et al., 2021). It is believed that, unlike other sensory systems, the connection between the hippocampus and the olfactory analyzer is quite close, which can be traced during the evolution of mammals (Allen and Fortin, 2013; Schwarz et al., 2013; Gass et al., 2014; Mechling et al., 2014; Liska et al., 2015; Lu et al., 2019). Meanwhile, during the evolution of placental mammals, the neocortex expanded, shifting the functional networks of the hippocampus from the primary sensory cortex to the association cortex (Buckner and Krienen, 2013). Thus, the functional networks of the human hippocampus predominantly include higher associative areas of the cortex, while connections with the primary sensory cortex are preserved in rodents (Bergmann et al., 2016). In the pHHC model, the olfactory function has not previously been considered. Integration between different parts of the brain is especially important in sense analysis (in particular, olfactory) and in organizing the motor activity of animals.

We studied most of the structural abnormalities in the pHHC model in the first month of postnatal ontogenesis, when homocysteine levels normalize and neuroinflammatory processes are noted, while information about changes at the adult stage is fragmentary, which makes microscopic and ultrastructural studies of hippocampal tissue in adult pHHC animals important.

Based on the above, the purpose of this work was to study neurodegenerative changes in the ultrastructural organization of the CA1 region of the dorsal hippocampus in adult rats that underwent pHHC, as well as behavioral disorders in animals associated with olfactory function.

MATERIALS AND METHODS

The work was carried out on Wistar rats from the Rappolovo nursery (St. Petersburg, Russia). When carrying out the work, we used a previously developed method for modeling hyperhomocysteinemia (HHC), based on a dosed methionine load created by forced oral administration of a 0.15% aqueous solution of L-methionine to experimental animals (0.10–0.15 g per animal, daily), starting from 4 days after fertilization until delivery (Harutyunyan et al., 2012). Female rats of the control group were additionally given water orally at the same time. Morphological and behavioral studies were carried out on males from the offspring of these females. Using light and electron microscopic techniques, a morphofunctional study of the structural organization of the CA1 region of the dorsal hippocampus of control rats and destructive changes in it in rats that underwent pHHC were carried out 3 months (stage P90) after birth.

Light Microscopy

Nissl Method

Morphological analysis of the state of brain nerve cells was carried out on other samples of animals: control and experimental rats at the adult stage of development, with nine animals in each group. After transcardial perfusion with 10% neutral formalin in phosphate buffer, the brain was cut on a Leica SM15105 cryostat (Leica, Germany), and the resulting 20-μm-thick sections of the dorsal hippocampus were stained with cresyl violet using the Nissl method.

Golgi Method

The study of the direction and nature of branching of dendritic processes, as well as of the location of spines and varicose dilations of dendrites, was carried out using the Golgi silver chrome method. Adult control rats ($n = 10$) were anesthetized with Zoletil (30 mg per 1 kg of weight) and decapitated. The brain was removed, and a block with telencephalon tissue containing the hippocampus was fixed. We used the classic method with 2-week osmation and silvering for up to 1 week. The procedure for preparing the material was carried out according to the protocol described in detail by us earlier (Belekhova and Tumanova, 1988). The brain was embedded in celloidin, and a series of frontal sections 75–100 μm thick were prepared.

Morphometric analysis was used to count varicosities and dendritic spines in the basal and apical dendritic zones of the CA1 region of the dorsal hippocampus. The number of animals in the control group sample was ten. For analysis, tissue sections of the *stratum oriens* ($n = 7$) and *stratum radiatum* ($n = 9$) were selected on which there were single dendritic trees of neurons, characterized by uniform impregnation with silver. Calculations of the average number of dendritic spines and varicosities were carried out on smooth, straight sections of dendrites from layers of the *stratum oriens* and *stratum radiatum*. For each region of interest in the animal's hippocampus, eight such dendritic sites were analyzed.

Ultrastructural Analysis

Ultrastructural analysis of nervous tissue of the brain was carried out in control and rats with pHHC at the adult stage of development, with two animals in each group. Animals were anesthetized as described above. To study intercellular relationships, the structure of the neuropil and the characteristics of synaptic contacts in the CA1 region of the dorsal hippocampus of rats, tissue was fixed for electron microscopic examination by transcardial perfusion with a mixture of 1% glutaraldehyde and 1% formaldehyde in 0.1 M PBS, pH 7.4. Next, the brain area with the hippocampus was fixed with 1% OsO_4, counterstained with uranyl acetate, dehydrated, and embedded in Epon according to the standard protocol (Shcherbitskaia et al., 2021). Ultrathin sections 50 nm thick were prepared using an LKB-III ultratome (LKB, Sweden), which were then examined using an FEI Tecnai G2 Spirit electron microscope (FEI, United States).

Immunofluorescence

For the purpose of comparative assessment of myelination at periods P20 and P90 in normal rats ($n = 9$) and at pHHC ($n = 9$) studied the distribution of the marker protein of myelin fibers—oligodendrocyte glycoprotein (GO) using immunofluorescent analysis. Primary antibodies ab24022 were used at a dilution of 1 : 1000) and FITC-conjugated secondary antibodies ab6785 at a dilution of 1 : 200 (Abcam, United States). We studied a section of the CA1 region of the dorsal hippocampus (4.5 mm from Bregma according to the atlas of (Paxinos and Watson, 2007)), calculating the average brightness of the glow in an area 500 μm wide, covering all layers of the *cornus ammoni*, from the *stratum oriens* to the *stratum radiatum*. At the same time, sections of layers were selected for analysis of the *stratum oriens* and *stratum radiatum* for quantitative analysis. The first sequence slice had a random location within the region of interest, the distance between subsequent ones was 40 μm. A double negative control system was used in the work. The GO^+—liver (negative control) was liver tissue from rats in the control group, on sections of which a full procedure of immunochemical staining of GO was carried out with both primary and secondary antibodies. In addition, a GO negative control was selected for each animal: adjacent sections of the hippocampus, in which the immunochemical reaction was carried out only with secondary antibodies in the absence of primary antibodies. Using the VideoTest Master Morphology program (VideoTest, St. Petersburg, Russia), the average brightness of the immunofluorescence of the visualized glycoprotein was measured in each analyzed area of the hippocampus (seven areas from each animal). Then, for each studied area, the difference between the measured average luminescence brightness in the area and the brightness of a similar field corresponding to the negative control was calculated, using it in further statistical processing as an index of myelination. Animals of the control group and with pHHC were compared according to the value of the obtained myelination index using the nonparametric Mann–Whitney test.

Odor Preference Test

Odor samples were presented to adult (P90) animals from the control and pHHC groups ($n = 15$ per group) daily for 8 days. The rat was placed in the center of the chamber (1000 × 1000 × 400 mm), and the number of times that a rat approached each open glass container with a drop of 0.1 mL of one of six essential oils at the bottom (30 mm in diameter and height) was recorded for 15 min. Containers with odorants were placed in a circle at the same distance from each other and from the walls of the chamber in which testing was carried out; their position was changed with each new presentation. After each animal, the surfaces of the chamber were wiped with a 50% solution of ethyl alcohol. For each rat, the Preference Index for each odor, averaged over all testing days, was calculated. Preference Index represented the number of approaches to the container of a particular odorant as a percentage of the total number of approaches to all containers with odorants. We used natural essential oils (cloves, mint, eucalyptus, wormwood, lavender, and valerian). Of the entire set of odors, only valerian had a functionally significant pheromonal effect common to different mammalian species (Müller-Schwarze et al., 1974; Matsumoto-Oda et al., 2003; Melnik et al., 2009). Statistical processing of the results was carried out using one-way ANOVA followed by Bonferroni post hoc analysis.

RESULTS

Neurodegenerative Changes in the CA1 Region of the Dorsal Hippocampus of Rats (P90) That Underwent pHHC

In the pyramidal layer of the CA1 region of the dorsal hippocampus of adult rats with pHHC, in contrast to the control, significant changes were found in the structure of neurons degenerating according to the chromatolytic type, with local disappearance of organelles in the cytoplasm. Chromatolysis was observed both at the light-optical level (Fig. 1b compared to Fig. 1a) and on electron diffraction patterns (Fig. 1d compared to Fig. 1c). We also noted chromatolysis in the early stages of development (Shcherbitskaia et al., 2021). In addition to chromatolysis, a neurofilamentous type of degeneration was also found in adult animals, in which the cytoplasmic organelles are completely replaced by neurofilaments (Fig. 1d compared to Fig. 1c). The release of these neurofilaments from the cell into the processes of neurons was often observed (Fig. 1f). Lysosomes and autophago-

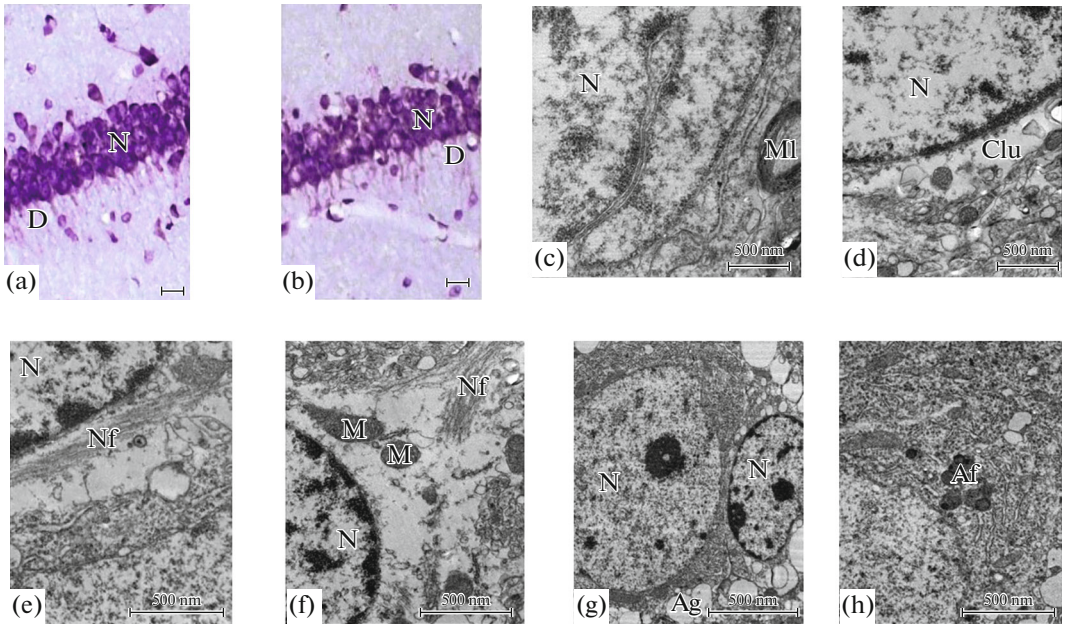

Fig. 1. Neurodegenerative changes in (b, d–i) cells of the pyramidal layer of the CA1 region of the dorsal hippocampus in adult rats that underwent pHHC in comparison with (a, c) control. (a, b) Microphotographs of the CA1 field of the hippocampus in (a) control rats and (b) rats that had undergone pHHC. Nissl staining, scale bar: 30 μm. Arrows indicate pyramidal neurons in a state of chromatolysis; N—neurons, D—dendrites. (c–i) Electron diffraction patterns of the CA1 region of the hippocampus of (c) control rats and (d–i) rats with pHHC; (d) chromatolysis (Chr), (e, f) neurofilamentous type of cellular degeneration, (g) release of neurofilaments into the neuron process, (h) activation of astrocytic glia, and (i) autophagosomes in the cytoplasm of a neuron are shown. Ml— myelinated fibers, M—mitochondria, Nf—neurofilaments, Ag—astrocytic glia processes, Af— autophagosomes.

somes are found in the cytoplasm of dying neurons in different quantities and with different forms (Fig. 1i) and activation of astrocytic glia is observed (Fig. 1h).

Compared with control animals, significant destruction of dendritic processes was found in adult rats with pHHC in the areas of basal and apical dendrites. A large number of cisterns appear in the cytoplasm of the axial cylinder of dendrites, the cristae of mitochondria are completely destroyed, the turgor of the axial cylinder of the dendrite decreases, and its shell becomes convoluted. These signs of pathology are also observed in the basal (Figs. 2d–2f) and in apical (Figs. 3a–3d) dendrites compared to control (Figs. 2a–2c). Dilated cisterns are also found in the places of dendrites from where spiny processes and spines extend (Figs. 3c, 3d). The same destructive phenomena can be observed in the structure of numerous basal dendritic varicosities (Fig. 2h) and apical dendrites (Figs. 3a, 3b). In Figs. 2h and 2i, small varicosities of the basal dendrites are shown. In their axial cylinders, large cisterns are visible in the cytoplasm (Figs. 2d–2f), as are destroyed mitochondrial cristae. The same signs of degenerative changes were found in the axial cylinders of the apical dendrites (Figs. 3a, 3b). Due to the smaller size of the distal sections of the basal dendrites compared to the apical dendrites, varicosities in the former are much smaller in size and are characterized by a large number of cisterns, occupying a significant volume of varicosities (Figs. 2h, 2i). The same signs of destruction are noticeable in axonal varicosities, in which an accumulation of synaptic vesicles occurs in the area of synaptic contacts (Figs. 2j, 2l). The spines are mainly located on the dendritic branches between the varicosities and have a variety of shapes: on a long thin stalk with a small head, on a thick stalk with a large head, or without a head (Figs. 3e, 3f).

To more fully understand the state of neural networks in the CA1 region of the dorsal hippocampus, we resorted to data obtained using the silver chrome Golgi method in control rats. According to these data, numerous, thin, and long basal dendrites up to 300 μm long with secondary and tertiary branches, with numerous spines on their surface, extend from the bodies of pyramidal neurons (Figs. 4a–4c). They are directed dorsally, perpendicular to the surface of the hippocampus. In the *stratum oriens* layer, basal dendrites change direction and move parallel to the dorsal surface of the hippocampus, heading towards the *tractus temporo-ammoni*. In this tract, numerous varicosities are noted on the branches of the basal dendrites and axons, and spiny dendritic spines are also visible. Powerful apical dendrites with numerous branches up to 500 μm long are directed from the bodies of pyrami-

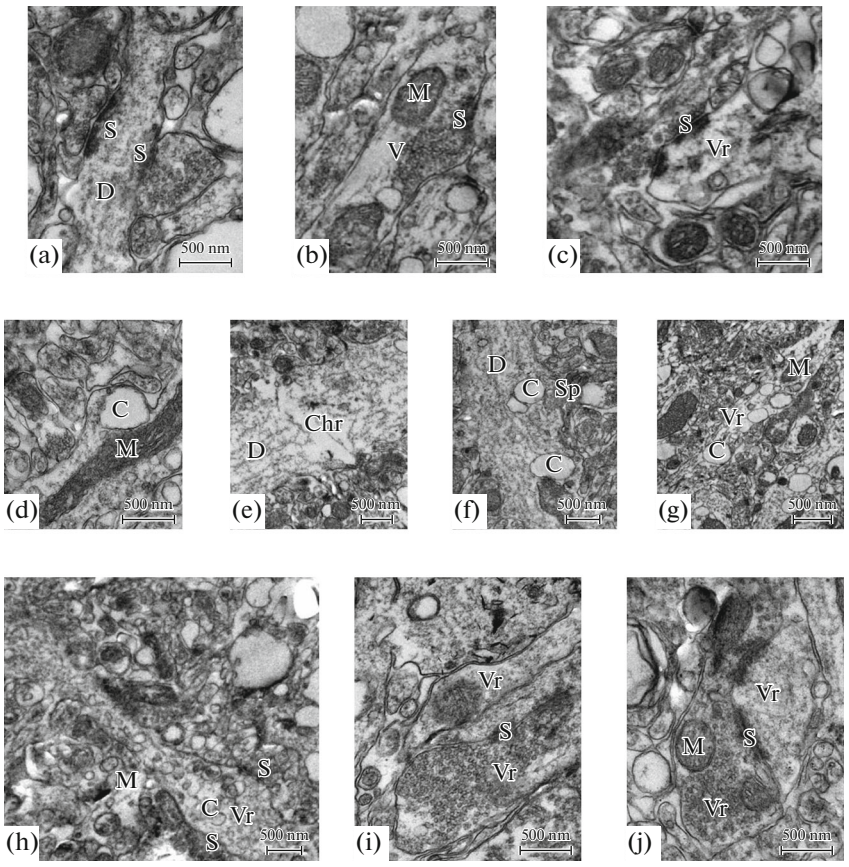

Fig. 2. Destructive changes in the basal dendrites of the CA1 region of the dorsal hippocampus. Electron diffraction patterns of (a–c) control rats and (d–j) rats with pHHC at the age of P90. CqQcisterns, CHR – chromatolysis, D—dendrites, M—mitochondria, N— neurons, S—synaptic terminals with contacts, Sp—dendritic spines, Vr—varicosities.

dal neurons ventrally into the layer *stratum radiatum*, where Schaffer collaterals pass. The surface of the dendritic processes is densely dotted with numerous dendritic spines and varicosities. On the dendritic branches of the second and third order, a dense network of varicosities is noticeable. In Fig. 4e, pyramidal neurons with numerous processes of apical dendrites are shown. In addition to numerous varicosities, a large number of dendritic spines are found on the distal portions of the dendrites.

Morphometric analysis showed that, in adult control animals, the average number of varicosities in the *stratum radiatum* per 1 μm of length of the dendrite section was 0.13 ± 0.01, while this number was 0.03 ± 0.01 in the *stratum oriens* (Fig. 4f). The distribution of dendritic spines was uneven (they were more numerous in the distal areas); the average number of dendritic spines per 1 μm was 0.10 ± 0.02 in the *stratum radiatum* and 0.09 ± 0.02 in the *stratum oriens* (Fig. 4e). In the proximal part of the dendrites, near the neuron bodies, spines were absent.

Myelination in Offspring of pHHC Rats

To identify possible causes of impaired axonal myelination in adult animals with pHHC, the distribution of GO was immunohistochemically studied during the period of active myelination of hippocampal fibers at P20 and P90.

In rats that underwent pHHC, neurodegenerative changes were observed in the CA1 region of the dorsal hippocampus compared to control. In the zones of the basal and apical dendrites of the CA1 region at the adult stage of development (P90), signs of destruction of myelinated fibers, which play an important role in conducting stimuli in the hippocampus, were found. In myelinated fibers, changes in axoplasmic turgor, destruction of mitochondrial cristae, the appearance of dilated cisterns in it and significant stratification of the myelin sheath in the form of convex pockets with twisted lamellae were found (Figs. 5a, 5b, 3a).

For comparison, at P20, rats with pHHC showed a disturbance in the process of myelination of nerve fibers in the CA1 region of the dorsal hippocampus. A study of normal GO distribution (Figs. 5e, 5i) and with pHHC (Figs. 5m, 5p) showed a difference in

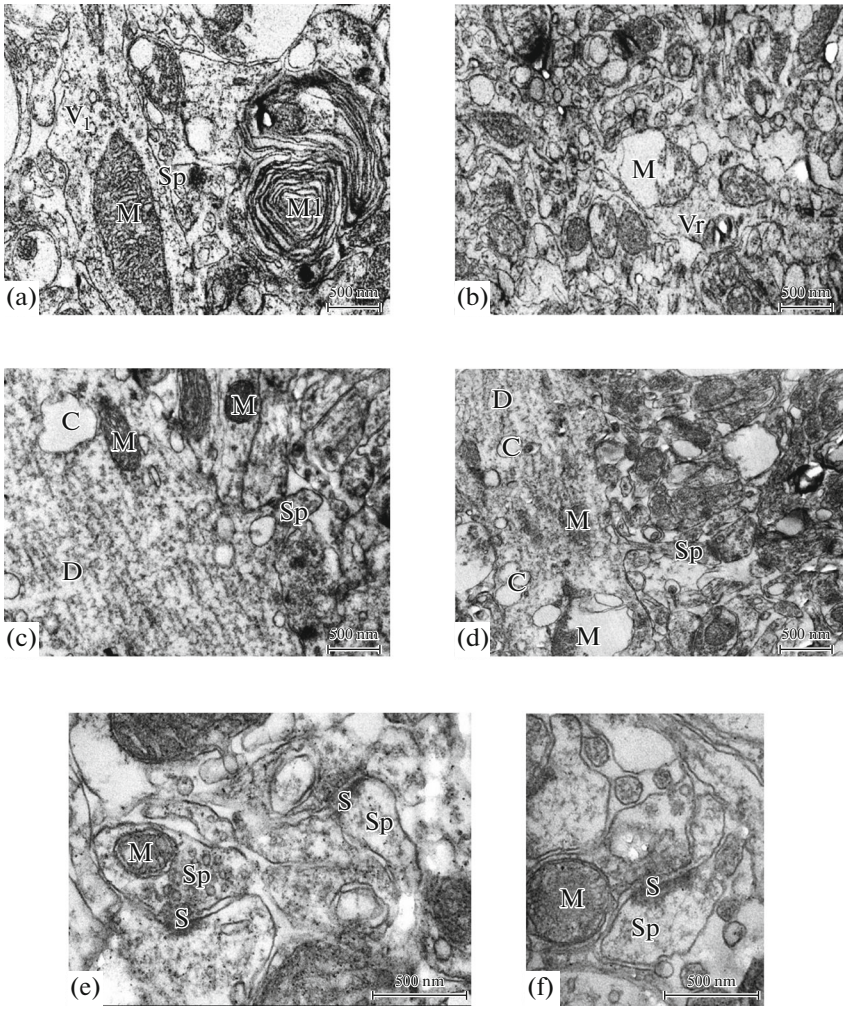

Fig. 3. Destructive changes in the apical dendrites of the CA1 region of the dorsal hippocampus. (a–e) Electron diffraction patterns of rats with pHHC at the age of P90. Ml—myelinated fibers, D—dendrites, M—mitochondria, Vr—varicosities, C—cisterns, S—synaptic terminals with contacts, Sp—dendritic spines.

myelination indices, which characterize the rate of myelination of nerve fibers. Thus, after prenatal pHHC this index in the *stratum oriens* was reduced by 21.2% relative to the control level (Mann–Whitney test, $U = 10$, $p = 0.005$; Fig. 5f), while in the *stratum radiatum* it was reduced by 21.4% (Mann–Whitney test, $U = 5$, $p < 0.001$; Fig. 5n). At the adult stage (P90), no statistically significant differences were found between the control and experimental groups, neither in the *stratum oriens* (Mann–Whitney test, $U = 20$, $p = 0.08$; Fig. 5j) nor in the *stratum radiatum* (Mann–Whitney test, $U = 26$; $p = 0.22$; Fig. 5r). In rat liver tissue lacking GO, there was no immunochemical reaction. The data obtained may indicate a lag in the process of myelination of afferent and efferent fibers in the CA1 region of the hippocampus, and may also indicate the development of the process of dissection of the myelin sheath in pHHC rat pups, which is consistent with the results of electron microscopy in adult animals.

Odor Preferences in Adult Control and pHHC Rats

The results of the study showed that rats who had undergone pHHC were more actively interested in odors: the average number of approaches to the container with essential oils in rats with pHHC was higher ($t = -2.42$, $p = 0.02$, Student's test for independent samples) than in control animals and was, respectively, 67.19 ± 5.99 versus 49.58 ± 4.14. Control animals gave the greatest preference to valerian oil (Fig. 6a): the number of approaches to the container with it prevailed over the other five ($F_{5;84} = 42.35$, $p < 0.0000$, one-way ANOVA analysis, Bonferroni post hoc test). Control rats approached the container with wormwood oil least often, but no statistically signifi-

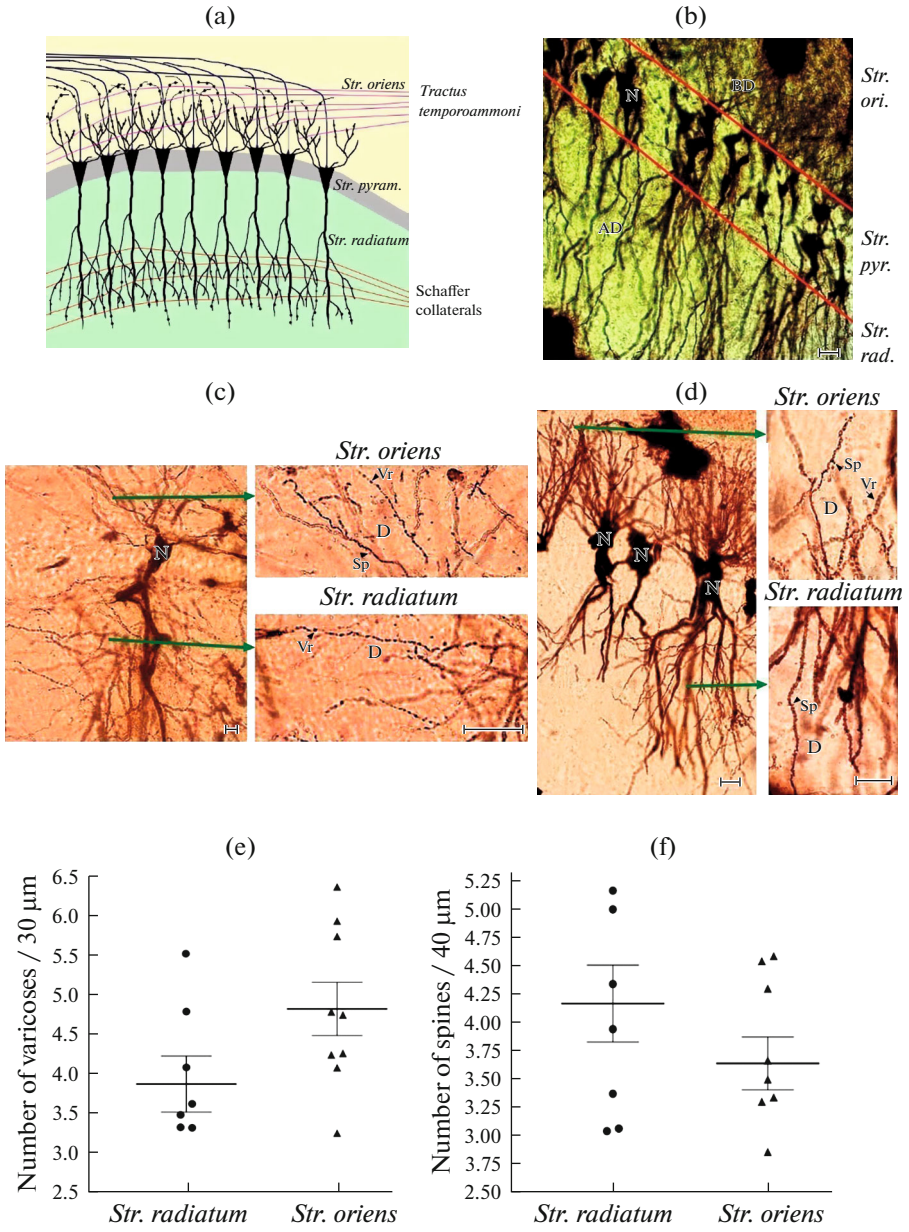

Fig. 4. Structural organization of basal and apical dendrites of pyramidal neurons in the CA1 region of the dorsal hippocampus of adult control rats. (a) Schematic representation of the cytoarchitecture of the CA1 region of the dorsal hippocampus of rats. (b–d) Photomicrographs of the CA1 region of the dorsal hippocampus of adult (P90) control rats; Golgi method, scale bar 10 µm; in the center, there is a layer of pyramidal neurons (*Str. pyramidale*). (*c*) Varicosities on the basal and apical dendrites of pyramidal neurons. (d) Powerful bundles of basal and apical dendrites with dendritic spines and varicosities. (e, f) Quantitative analysis of (e) varicosities and (f) dendritic spines in the area of basal (*Str. oriens*) and apical (*Str. radiatum*) dendrites of pyramidal neurons in control rats; the average values and their errors are shown. Designations: *Str.*—*stratum*, N—neurons, AD—apical dendrites, D—dendrites, BD—basal dendrites, Vr—varicose dendrites, Sp—dendritic spines.

cant difference was found in the number of approaches to other odors except valerian. Attention to the smell of valerian for rats with pHHC was at the level of the other four odorants – clove, mint, eucalyptus and lavender, with the exception of the smell of wormwood (Fig. 6b), characterized by the smallest number of approaches to the container with this odorant. The number of approaches to a container with the smell of wormwood was statistically significantly different from that for valerian and cloves ($F_{5;84} = 3.42$, $p = 0.039$ and 0.006, respectively: one-way ANOVA, Bonferroni post hoc test) compared to control animals. rats that underwent pHHc approached the container with valerian oil 1.5 times less often ($t = 6.19$, $p < 0.0001$, Student's t-test for independent samples). Since it is believed that valerian oil has pheromonal

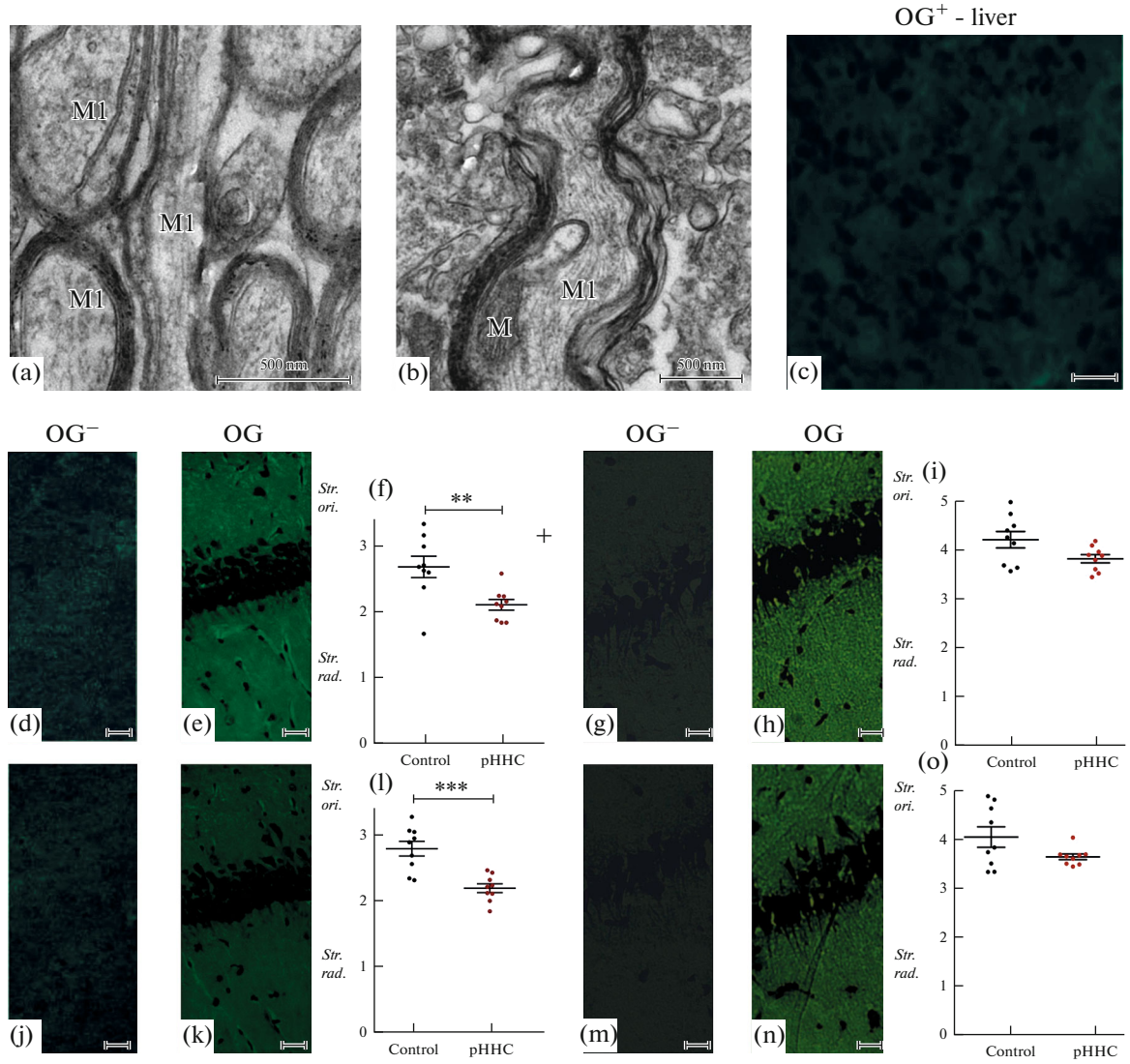

Fig. 5. The effect of pHHC on the myelination of nerve fibers in the dorsal hippocampal field of rats. (a, b) Electron diffraction patterns of (a) control rats and (b) rats with pHHC at the age of P90. M—myelinated fibers, M—mitochondria. (c–o) Distribution comparison oligodendrocyte glycoprotein (GO, FITC fluorescence) in (c) liver tissue and (d, e, h, i, l, m, o, p) CA1 region of the hippocampus of (d, h, i) control rats and rats (l, m, o, p) with pHHC at the ages of (d, e, l, n) P20 and (h, i, o, p) P90. Immunohistochemical staining, scale bar 20 μm. (*c*) negative control GO^+–liver (complete immunochemical reaction on a liver tissue preparation from a control rat at P20) with primary and secondary antibodies; scale 40 μm. (d, h, l, o) Negative control (GO^-—immunochemical reaction on a hippocampal tissue preparation in the absence of primary antibodies). (f, j, n, o) Myelination index, arb. units (difference in luminescence brightness between the tissue area under study and the corresponding negative control GO^-)—results of densitometry of immunochemical staining of GO in the *stratum oriens* layer (*Str. ori.*: f, j) and in *stratum raditum* (*Str. rad.*: n, o) layer in rats at (f, n) P20 and (j, o) P90; data are presented as the mean and its error; asterisks indicate differences between the control group and pHHC at P20 at $^{**}p = 0.005$ and $^{***}p = 0.0008$ (nonparametric Mann–Whitney test).

properties and is normally a physiologically significant stimulus, we can conclude that pHHC has a negative effect on the functioning of the olfactory analyzer.

DISCUSSION

When embryonic development is disrupted, in particular with pHHC, destructive changes in the ultrastructural organization of the neural networks of this important section were found in the CA1 region of the dorsal hippocampus of rats. We have shown that, after pHHC in early ontogenesis (P5), in all CA1 zones of the dorsal hippocampus, rats showed signs of a lag in the maturation and a development of the main elements of nervous tissue compared to control animals: a large volume of intercellular space, a large number of growth cones, developmental delay dendritic, and axonal processes and insufficient development of organelles in the cytoplasm of neurons (Shcherbitskaia et al., 2021; Vasilev et al., 2023).

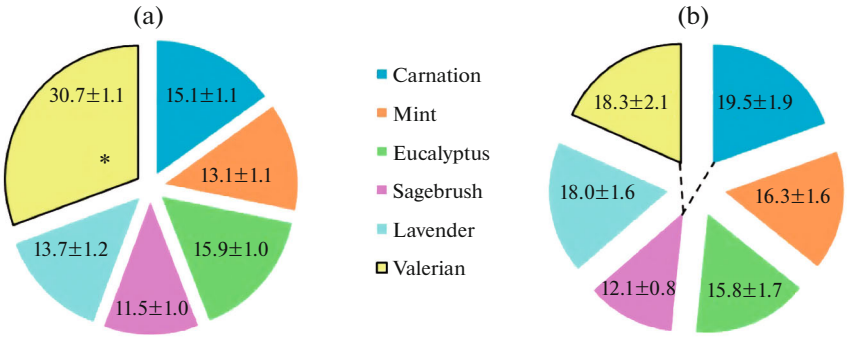

Fig. 6. Scheme of distribution of odorant odor preference indices in (a) adult control rats and (b) rats with pHHC. The indices are represented by multicolored sectors and expressed by the number of approaches (average value and its error) to a container with the corresponding natural vegetable oil odorant in % of the total number of approaches to all containers. The black border marks the sector corresponding to the physiologically significant smell of valerian. Dashed lines between sectors indicate statistically significant differences in preference indices between odorants in animals with pHHC. Volume of each sample $n = 15$. One-way ANOVA followed by Bonferroni post hoc analysis. * Preference for the smell of valerian relative to other five smells is significant when $p < 0.0001$; # preference for the smell of wormwood relative to cloves, as well as the smell of wormwood relative to valerian, is reliable when $p < 0.05$.

In this study, we investigated the effect of homocysteine on changes in the ultrastructural organization of the hippocampus of pHHC rats in a later period of ontogenetic development - in adult rats at P90. It was found that the majority of degenerating neurons of the pyramidal layer of CA1 are subject not only to chromatolysis, but also to the filamentous type of degeneration and are surrounded by numerous processes of activated astroglia. A large number of autophagosomes and lysosomes were found in the cytoplasm of degenerating neurons. It has been shown that increased levels of proinflammatory cytokines indicate a neuroinflammatory response in the hippocampus of adult rats after pHHC, which was also observed at earlier stages of ontogenesis (Shcherbitskaia et al., 2021).

In the areas where the basal and apical dendrites are located, neurodegenerative changes occur in the pathways: disintegration of myelinated fibers, in addition to destruction of the basal dendrites (in the *stratum oriens* layer) and apical dendrites (in the *stratum radiatum* layer), as well as in dendritic varicosities, which are located in large numbers on the distal portions of these dendrites directed into the tracts of the *tractus temporoammoni* and Schaffer collaterals. According to electron microscopy data, degenerative changes in these varicosities do not occur in control animals.

In our earlier studies on the effect of homocysteine on the formation of parts of the rat brain, it was shown that, at the early stages (P5) of postnatal development of rats, the myelin sheath of axonal fibers in CA1 has not yet been formed (Shcherbitskaia et al., 2021). However, at P20, we noted a decrease in the immunochemical reaction to GO in CA1 of the dorsal hippocampus, which may indicate a delay in the process of axonal myelination in rat pups with pHHC and disruption of the myelin layers. GO (myelin oligodendrocyte glycoprotein) is an important marker of myelination. It is localized both on the surface of oligodendrocytes and in all myelinated fibers of the hippocampus and is involved in the stabilization and integration of myelin layers. Its role in the pathogenesis of multiple sclerosis has been shown (Berger et al., 2003). It is known from the literature that GO is involved in the stabilization of myelin layers and is considered as a promising marker for identifying demyelination in experimental models and diagnosing demyelinating diseases (Kitley et al., 2012). Its insufficient expression or neutralization by specific antibodies (Berger et al., 2003; Kitley et al., 2012) causes disintegration (separation) of the myelin layers, similar to that in multiple sclerosis (Ketelslegers et al., 2015), autoimmune encephalomyelitis (Kezuka et al., 2012), and our observations in the present study. Data on changes in the distribution of GO at P20 are consistent with the results on the disintegration of myelin layers in adult animals described in this work.

Compared with control rats, all of the listed signs of degenerative changes in neurons of the pyramidal layer, myelinated fibers, unmyelinated axons, dendrites, and varicosities of the basal dendritic zone and apical dendritic zone indicate the toxic effect of homocysteine and a serious disruption of the structure of nervous tissue in the CA1 region of the dorsal hippocampus of rats who have undergone pHHC, as well as indicating the possibility of a decrease in the plasticity of this important part of the brain, which is reflected in the behavior of animals.

We have previously shown that pHHC disrupts learning and memory in adult animals (Postnikova et al., 2022). Since the hippocampus is one of the links in the olfactory analyzer, in the present work we investigated the effect of the pHHC on the sense of smell and found that the pHHC is able to influence the

behavior of adult rats associated with the olfactory function. Normally, among the six odors of natural essential oils, the most attractive for all control rats was valerian oil. This result is consistent with our data described previously (Dubrovskaya et al., 2022) and is quite logical, since the components of valerian oil—in particular, isovaleric acid—have pheromonal significance for this animal species and are widely used in the study of the olfactory function in rodents (Boryakova et al., 2007; Melnik et al., 2009, 2012). In adult rats, pHHC completely neutralized the preference for the smell of valerian, which is normally a physiologically significant stimulus, and so we can conclude that pHHC has a detrimental effect on the normal functioning of the olfactory analyzer.

In adult control rats, using the Golgi method, we showed that, upon entering the *temporammonic* tracts and Schaffer collaterals, a dense network of varicosities appears on the surface of the distal segments of the basal and apical dendrites.

Morphometric analysis carried out at the light-optical level showed that the number of dendritic spines in the *stratum radiatum* is greater than in the *stratum oriens* and there are more dendritic varicosities in the *stratum oriens* than in the *stratum radiatum*. We previously observed similar signs of entry into the tracts of nerve fibers and dendrites with varicosities in the thalamus and amygdala of reptiles, where integrative connections were also carried out within various sensory systems (Belekhova and Tumanova, 1988). The authors noted the similarity of the principles of brain organization that carry out such integrative activity in animals in the evolutionary series of vertebrates (Belekhova and Tumanova, 1988). Based on the obtained facts, one can assess the specificity of the perception of multimodal impulses and draw a fundamentally important conclusion regarding the emergence of other adaptive systems in a number of vertebrate animals. This fact of entry into tracts of dendrites with varicosities indicates an increase in the specialized surface for axodendritic synapses. According to Belekhova's ideas about the peculiarities of the organization and structure of the brain regions of lower vertebrates, the origins of heterosensory convergence are based on phylogenetically ancient mechanisms of afferentation of neural networks of the brain in lower vertebrates. Therefore, the progressive specialization of sensory centers in the evolution of vertebrates occurred through the transformation of these systems (Belekhova and Tumanova, 1988).

The formation of a dense network of varicosities on the dendritic processes of CA1 indicates the specialized construction of neural networks with a significant increase in the contact synaptic surface with multifunctional fibers directed to the hippocampus. The signs that we observed of neurodegenerative changes in the pathways in the CA1 region of the dorsal hippocampus of rats with pHHC in early ontogenesis and in adult rats, as well as impaired behavior, indicate a possible change in the construction of neural networks. The literature indicates an increased sensitivity of sensory systems and cognitive functions to the action of damaging factors (Rice and Barone, 2000). Disruption of these functions is observed during the development of various neurodegenerative processes, both in the case of aging (the development of neurodegenerative diseases (Ribaut-Barassin et al., 2003)) and in the offspring of animals with various complications of pregnancy (Rice et al., 2000).

Thus, we can conclude that pHHC affects the tissue structure of the CA1 region of the dorsal hippocampus of rats, which is an important integrative center involved in the processing of multimodal stimuli. The action of pHHC caused destructive changes in nervous tissue, which we discovered in the CA1 region of the dorsal hippocampus not only in early ontogenesis, but also at the adult stage. The observed disturbances can have a detrimental effect on the functioning of sensory systems, as well as on cognitive functions.

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AUTHOR CONTRIBUTION

N. Tumanova: work idea and experiment planning, writing and editing text. N. Tumanova, D. Vasiliev, N. Dubrovskaya: data collection and processing. Text and figures were approved by all coauthors.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All studies were conducted in accordance with the principles of biomedical ethics as set out in the 1964 Declaration of Helsinki and its subsequent amendments. They were also approved by the Ethics Committee of the SIEPB Institute of Evolutionary Physiology and Biochemistry (St. Petersburg, Russia), protocol no. 3/2020 dated March 18, 2020.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest

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