

## CYTOCHEMICAL FEATURES OF OLFACTORY RECEPTOR CELLS IN BENTHIC AND PELAGIC SCULPINS (COTTOIDEI) FROM LAKE BAIKAL

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**Abstract:** Electron and laser confocal microscopy were used to analyze the adaptive cytochemical features of the olfactory epithelium in three genetically close deep-water Cottoidei species endemic to Lake Baikal – golomyanka (Baikal oilfish) *Comephorus baicalensis*, longfin Baikal sculpin *Cottocomephorus inermis* and fat sculpin *Batrachocottus nikolskii* – whose foraging strategies are realized under different hydrostatic pressure regimes. Hypobaric hypoxia that developed in *B. nikolskii* (a deep-water benthic species) upon delivery to the surface caused distinct destructive changes in cells of the olfactory epithelium. In *C. baicalensis* and *C. inermis*, whose foraging behavior involves daily vertical migrations between deep and shallow layers, these cells are characterized by a significantly higher structural and functional stability than in deep-water *B. nikolskii*. The results of morphological study and quantitative analysis of functionally active mitochondria in cells of the olfactory epithelium of closely related deep-water fish species with different modes of life provide evidence that tolerance of the olfactory apparatus to hypobaric hypoxia is different in pelagic and benthic species. These results help elucidate the mechanisms responsible for the consistent functioning of the olfactory system in animals evolutionarily adapted to extreme environmental factors, and provide theoretical and practical implications in different fields of biology, neurology and extreme medicine.

**Key words:** olfaction; neuron; hydrostatic pressure; adaptation; ultrastructural alterations; hypoxia

### INTRODUCTION

Analysis of the processes involved in the perception of biologically significant olfactory signals is one of the key areas of interest in modern neurobiology and medicine [1,2]. In particular, this concerns the mechanisms of olfactory receptor cell functioning, not only in environments with different chemical characteristics but also under conditions of exposure to a wide range of hydrostatic pressures. Such studies are especially relevant in certain hydrobionts, which, in the course of evolution, have adapted to living under

different hydrostatic pressure regimes. To date, numerous studies have been published dealing with the structural and functional organization of the olfactory system in fishes from different taxonomic groups and with different behavioral patterns [3-6]. However, deep-water species usually outlie the scope of relevant publications because of difficulties in catching them. Hence, data on the level of development of morpho-functional systems in such species are scarce [7-9], and those on the olfactory system are usually limited to general anatomical characteristics [10,11].

This study is devoted to analysis of the peripheral olfactory apparatus in genetically close sculpins (Cottoidei), endemic to Lake Baikal, which, in the course of evolution, have occupied separate ecological niches at different depths and developed distinct behavioral strategies. The evolutionary kinship of these species has been confirmed in comparative morphological and genetic studies [12,13]. In addition to electron microscopy, we have used cytochemical methods to analyze cellular elements of the sensory epithelium for the level of structural and functional integrity of mitochondria, which are known to be most vulnerable to hypoxia [14]. Studies on the olfactory system of animals living under extreme deep-water conditions will help to gain an insight into the cytological and molecular mechanisms allowing them to acquire tolerance to hypoxia and, consequently, to maintain the pattern of chemosensory behavior under variable hydrostatic pressure regimes.

Because of major technical difficulties in collecting biological material from large depths, we could analyze tissues of the olfactory apparatus only after the fish were delivered to the surface. Obviously, cells of the olfactory epithelium in these fish could be damaged due to hypobaric hypoxia developing upon the rapid transfer to a low-pressure environment [15]. Under such conditions, the adaptive properties of the olfactory system in deep-water fish species could be evaluated from different aspects: we could compare the degrees of intracellular lesions resulting from depressurization in fishes with different modes of life and, on the other hand, reveal the most stable ultrastructural characteristics of olfactory cells that have adaptive significance for deep-water fishes in their natural environment. In our opinion, such characteristics should remain largely unchanged upon depressurization.

## MATERIALS AND METHODS

### Animals

The study was performed on three species of deep-water Baikal sculpins: the golomyanka (Baikal oilfish) *Comephorus baicalensis* (Pallas, 1776), longfin Baikal

sculpin *Cottocomephorus inermis* (Jakowlew, 1890), and fat sculpin *Batrachocottus nikolskii* (Berg, 1900). *Comephorus baicalensis* (Fig. 1a) is a bladderless pelagic species that does not form schools and prefers depths from 100 to 750 m but also occurs in the bottom water layer (to depths of about 1600 m). These fish usually ascend to the upper water layers only at night, following the movements of their preferred prey (pelagic amphipods *Macrohectopus branickii* and larvae of their own species and *Comephorus dybowski*), and descend again during the day. Thus, *C. baicalensis* performs daily vertical migrations and is exposed to a wide range of hydrostatic pressures. *Cottocomephorus inermis* (Fig. 2a) is a bladderless benthopelagic species that occurs at depths of 10-15 to 1000 m and can also move between water layers with different hydrostatic pressures during the day. In summer, these fish live separately at depth of 300-1000 m; in winter they perform foraging migrations, rising to a 50-m water layer. The diet of *C. inermis* is similar to that of *C. baicalensis* and consists mainly of *Macrohectopus* amphipods and larvae of pelagic Cottoidei. *Batrachocottus nikolskii* (Fig. 3a) is a deep-water benthic species inhabiting mud-stone bottom areas at depths of 100-200 to more than 1500 m in the open-water zone of Baikal. Due to neutral buoyancy, *B. nikolskii* can swim up from the bottom and feed on pelagic fish, but its main prey are benthic amphipods and sculpins [13]. All manipulations with fish were carried out in compliance with the Helsinki Declaration of the World Medical Association (2000) and the EEC Directive 86/609 EEC (1986) on the protection and welfare of experimental animals. All experimental procedures were approved by the Bioethics Committee at the Scientific Council of the Department of Biology at Irkutsk State University 30.11.2007 year (Permit Number 3).

### Sampling

Microscopic and cytochemical analysis of olfactory rosettes was performed in three Baikal sculpins (*C. baicalensis*, *C. inermis* and *B. nikolskii*) occurring down to 1640 m (the maximum depth of the lake). One specimen of *B. nikolskii* was caught in July 2009 from aboard the *MIR-2* manned submersible at the bottom off the

Svyatoi Nos Peninsula (depth 1140 m) and delivered to the surface during the following 5 h. The olfactory epithelium of this fish was analyzed by transmission electron microscopy. Other specimens of all three species were caught in southern Baikal at depths of 220–260 m with nets set under the ice in March–April 2008–2014. Their delivery to the surface took 30 min. Ten specimens of each species were analyzed by methods of electron and laser confocal microscopy (Table 1).

**Table 1.** Morphometric, weight and sexual features of studied sculpins (Cottoidei) from Lake Baikal.

Species	n	L total, mm	Q, g	sex	age (years)
<i>C. baicalensis</i>	10	190–198	30.4–38.6	♀	5+ - 6+
<i>C. inermis</i>	10	154–161	29.5–44.1	♀	4+ - 5+
<i>B. nikolskii</i>	10	187–199	93.2–141.0	♀	6+ - 7+

### Transmission electron microscopy

The olfactory rosettes were fixed in 2.5% glutaraldehyde (Sigma-Aldrich, United States) solution in 0.1 M phosphate buffer, pH 7.3 at 4°C for 2 h, postfixed in 2% OsO<sub>4</sub> (Merck, Germany) in the same buffer for 12 h, dehydrated in an ascending alcohol gradient with acetone and embedded in Araldite 502 resin with a DMP-30 accelerator (Araldite 502 Kit, SPI Supplies, United States). Ultrathin sections (70–80 nm) made with an Ultracut R microtome (Leica, Germany) were examined under a Leo 906E transmission electron microscope at an accelerating voltage of 80 kV. Microscopic images made with a MegaView II digital camera were processed using the MegaVision program package (Soft Imaging System GmbH, Germany).

### Laser confocal microscopy

To objectively assess the functional state of mitochondria, we analyzed total preparations of olfactory rosettes. The rosettes were washed with medium 199 (Sigma-Aldrich) to remove blood and other contamination, stained for active mitochondria by incubating in 25 nM MitoTracker<sup>®</sup> Orange (Life Technologies, United States) in medium 199 for 25 min, and fixed in 2% paraformaldehyde solution for 15 min at 4°C. Cell nuclei were stained with 0.5 µg/mL DAPI solution

(Sigma-Aldrich) for 15 min. The preparations were then covered with the ProLong<sup>®</sup> Gold antifade reagent (Life Technologies) and analyzed under an LSM 710 laser confocal microscope (Carl Zeiss). The Z-stack images of the olfactory epithelium were processed with the Imaris<sup>®</sup> Bitplane 7.2.3 program package to calculate the number of mitochondria per  $1 \times 10^6 \mu\text{m}^3$  tissue volume. The results were processed statistically by non-parametric methods, and intergroup differences were assessed by the Kruskal-Wallis test, using Statistica 10 and Microsoft Excel 2010 program packages.

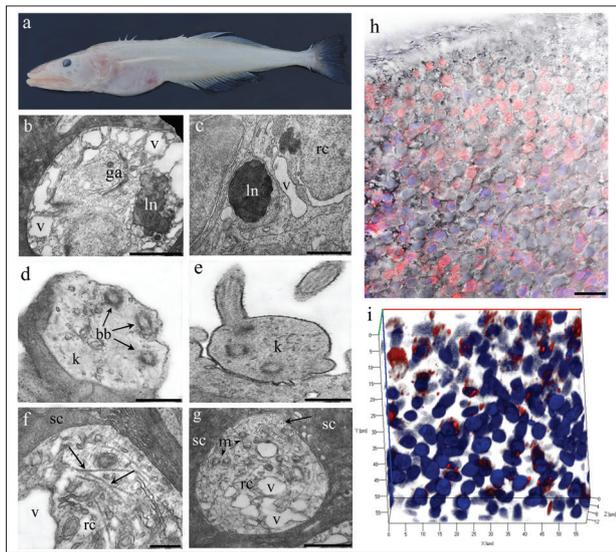
## RESULTS

The structure of olfactory rosettes is similar in all three species of Baikal sculpins: they consist of 5–6 folds (lamellae) lined on both sides with the receptor (chemosensory) epithelium, except for the non-sensory epithelium at their margins. As in other vertebrates, the chemosensory epithelium consists of three basic cell types: receptor, supporting and basal. The olfactory epithelium of Baikal sculpins includes receptor neurons of ciliated and microvillar types, the former prevailing in number.

### *Comephorus baicalensis*

The cytoplasm of supporting cells was electron dense and the intracellular components were poorly visible. The ultrastructural characteristics of receptor cells were indicative of low functional activity. Many of them had an electron-lucent light cytoplasmic matrix with sparse organelles and, hence, clearly stood out against the background of electron-dense supporting cells. The perinuclear space in some olfactory neurons was expanded all over the perimeter, which was a symptom of degenerative changes in the cell. The Golgi apparatus in the majority of receptor cells was inactive. It was located near the nucleus and consisted of stacked disk-shaped membrane sacks with a few small vesicles up to 0.15 µm in diameter. Lipofuscin granules and sparse, mostly free ribosomes were often found in the cytoplasm (Fig. 1b, c). A characteristic feature of the receptor cells is that they contained numer-

ous vacuole-like structures with irregular edges and electron-lucid contents. The size of these structures in different cells ranged from 0.2 to more than 2  $\mu\text{m}$ . Apparently, they were not necessarily derived from the Golgi apparatus but often appeared due to fragmentation and vacuolation of nearby canals of the endoplasmic reticulum (ER). Such vacuoles sometimes adjoined each other and occupied almost all free cytoplasmic space around the nucleus. In some neurons, such vacuoles occurred not only in the cell body but also throughout the dendrite, including the cell apex. However, the vacuoles were not incorporated into the cell membrane but remained in the apical cytoplasm. The olfactory knob in such vacuolated receptor cells usually had a low electron density and lacked structurally complete basal bodies and cilia or microvilli (Fig. 1d).



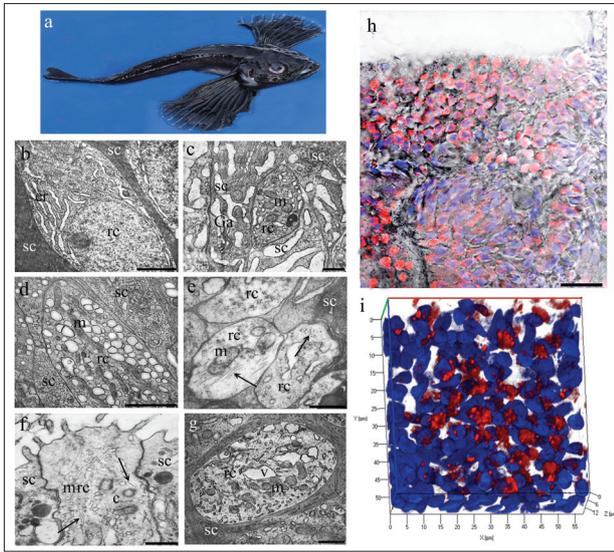
**Fig. 1.** Cytochemical features of the olfactory epithelium in the golomyanka, *Comephorus baicalensis*. (a) The golomyanka, exterior view. Transmission electron microscopy: (b) section through the perinuclear zone of a receptor cell with vacuoles in the cytoplasm; (c) a fragment of receptor cell cytoplasm with vacuoles and a lipofuscin granule; (d) the olfactory knob of a ciliated receptor cell with signs of degenerative changes; (e) an “intact” apex of ciliated receptor neuron; (f) longitudinal and (g) transverse neurofilaments in a dendrite; arrows indicate microfilaments. Confocal microscopy: (h) a fragment of olfactory epithelium in transmitted light; (i) a fragment of the olfactory epithelium, Z-stack image. Cell nuclei were stained with DAPI (blue), active mitochondria with MitoTracker<sup>®</sup> Orange (red). Designations: rc – receptor cell, ga – Golgi apparatus, sc – supporting cell, v – vacuole, m – mitochondria, ln – lipofuscin, bb – basal body, k – olfactory knob. Scale bars: (b), (c) – 2  $\mu\text{m}$ ; (d)-(f) – 0.5  $\mu\text{m}$ ; (g) – 1  $\mu\text{m}$ ; (h) – 15  $\mu\text{m}$ .

It should be noted that some receptor neurons in the olfactory epithelium showed similar degenerative changes but still retained well-defined chemosensory structures in the apical part (Fig. 1e).

Despite the poor development of intracellular organelles, receptor neurons contained well-defined cytoskeletal components, microtubules in particular. Dense microtubule bundles extended along the dendrite, amid vacuoles and mitochondria, to the olfactory knob (Fig. 1f). As could be seen in cross sections through the dendrites, there were also single neurofilaments extending in different directions, which occurred throughout the dendrite length (Figs. 1f, 1g). The cell bodies and dendrites of olfactory neurons contained relatively low numbers of small (0.2-0.3  $\mu\text{m}$ ) round or elongated mitochondria with sparse cristae, but without any signs of swelling or degeneration. Confocal microscopy of sections treated with MitoTracker<sup>®</sup> Orange, which stains only functionally active mitochondria, provided evidence for a high degree of preservation of these organelles (Figs. 1h, 1i), which was further confirmed by statistical analysis of the results of these cytochemical experiments (Fig. 4a).

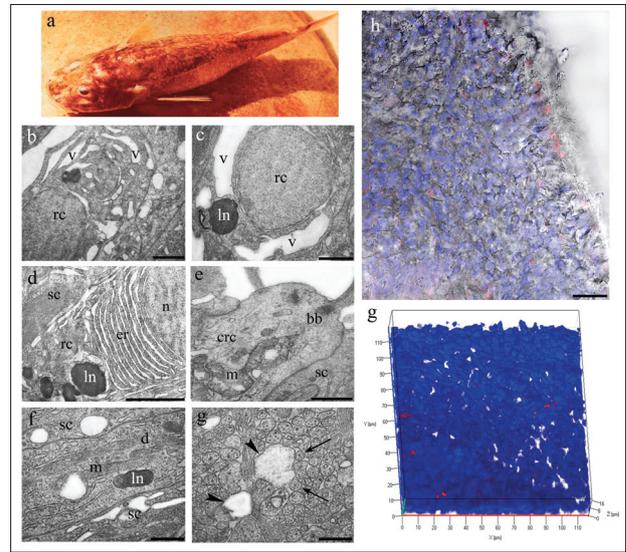
### *Cottocomephorus inermis*

As in *C. baicalensis*, the cytoplasm of supporting cells in *C. inermis* usually had increased electron density and relatively electron-lucid cell bodies, and dendrites of the olfactory neurons clearly stood out against this background (Fig. 2b). The level of organelle development in sensory cells was also similar to that in *C. baicalensis*. In the electron-lucid cytoplasmic matrix, an inactive Golgi apparatus and dilated ER canals with signs of vacuolation could be seen (Fig. 2c). The dendrites contained numerous vacuoles of different diameters (0.1-0.7  $\mu\text{m}$ ) with electron-lucid contents. Numerous vesicles of similar diameter and electron density were also detected in supporting cells, mainly those located in the upper layers of the olfactory epithelium (Fig. 2d). Analysis of sections made in different planes showed that the dendrites of receptor neurons contained well-developed microtubules and mitochondria (Figs. 2e, 2f). A noteworthy fact



**Fig. 2.** Cytochemical features of the olfactory epithelium in the longfin Baikal sculpin, *Cottomephorus inermis*. (a) The longfin sculpin, exterior view. Transmission electron microscopy: (b) the cell body of a receptor neuron with dilated ER canals; (c) cytoplasmic vacuolation in receptor and supporting cells; (d) receptor cell dendrite surrounded by supporting cells with numerous vesicles; (e) cross section through receptor cell dendrites (cytoskeletal elements and mitochondria are indicated); (f) the apex of a microvillar receptor cell with preserved microvilli (cytoskeletal elements are indicated); (g) cross section through receptor cell dendrite with a large vacuole and well-preserved mitochondria. Confocal microscopy: (h) a fragment of olfactory epithelium in transmitted light; (i) a fragment of olfactory epithelium, Z-stack image. Cell nuclei were stained with DAPI (blue), active mitochondria with MitoTracker<sup>®</sup> Orange (red). Designations: rc – receptor cell, mrc – microvillar receptor cell, sc – supporting cell, er – endoplasmic reticulum, v – vacuole, m – mitochondria, c – centrioles; arrows indicate microfilaments. Scale bars: (b) – 2 μm; (c) – 0.5 μm, (d) – 2 μm, (e-g) – 0.5 μm; (h) – 15 μm.

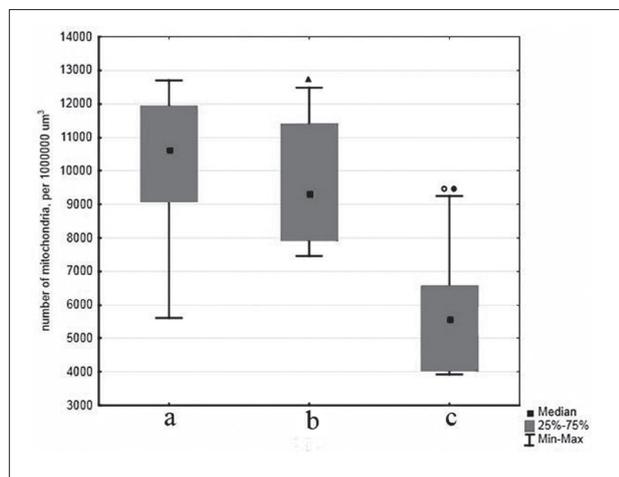
is that receptor cells with very large vacuoles occupying a major part of dendrite volume could often be detected amid electron-dense supporting cells in some areas of the olfactory epithelium. Moreover, it should be emphasized that, despite strong vacuolation, mitochondria located in the remaining cytoplasmic regions proved to retain their structure: they had a typical shape and contained sparse cristae and single glycogen granules (Fig. 2g). The results of confocal microscopy (Figs. 2h, 2i) and statistical analysis (Fig. 4b) confirmed the high functional stability of mitochondria in large areas of the olfactory epithelium.



**Fig. 3.** Cytochemical features of olfactory epithelium in the fat sculpin, *Batrachocottus nikolskii*. (a) The fat sculpin, exterior view. Transmission electron microscopy: (b) section through the perinuclear zone of a vacuolated receptor cell with a high density of ribosomes; (c, d) receptor cells with dilated ER canals and large lipofuscin granules; (e) the apex of a ciliated receptor cell with vacuolated mitochondria; (f) a fragment of receptor cell dendrite with a lipofuscin granule and mitochondria with signs of vacuolation; (g) “intact” (arrows) and vacuolated (arrowheads) axons of olfactory neurons in basal regions of the olfactory epithelium. Confocal microscopy: (h) a fragment of olfactory epithelium in transmitted light; (i) a fragment of olfactory epithelium, Z-stack image. Cell nuclei were stained with DAPI (blue); active mitochondria, with MitoTracker<sup>®</sup> Orange (red). Designations: rc – receptor cell, n – nucleus, er – endoplasmic reticulum, v – vacuole, sc – supporting cell, d – dendrite, m – mitochondria, l – lipofuscin. Scale bars: (b-d) 1 μm; (e-g) 0.5 μm; (h) 20 μm.

***Batrachocottus nikolskii***

Unlike those in pelagic sculpins, the receptor cells of the olfactory epithelium in *B. nikolskii* contained a well-developed Golgi apparatus and abundant ribosomes, both free and bound to ER, which was evidence for a high functional activity of these cells (Fig. 4b). The canals of the granular ER in both receptor and supporting cells were often fragmented and vacuolated, probably under the effect of acute hypoxia caused by the abrupt change in hydrostatic pressure when the deep-water fish were lifted to the surface.



**Fig. 4.** Numbers of functionally active mitochondria per  $1 \times 10^6 \mu\text{m}^3$  of olfactory epithelium in (a) *C. baicalensis*, (b) *C. inermis*, and (c) *B. nikolskii*: (▲)  $p=1.000$  compared to *C. baicalensis*; (○)  $p=0.047$  compared to *C. baicalensis*; (●)  $p=0.119$  compared to *C. inermis*.

A characteristic feature of cells in the olfactory epithelium of *B. nikolskii* was that they contained numerous lipofuscin granules. In the receptor neurons, they were usually located both in the cell body and in different dendrite segments. As a rule, these were electron-dense granules of irregular shape with an average size of 0.2-1  $\mu\text{m}$ . Lipofuscin also aggregated into large cytoplasmic bodies comparable in size to the cell nucleus. Analysis of olfactory rosette sections in different planes showed that the total volume of lipofuscin in the organ was significant, with this pigment being equally detected in the cytoplasm of receptor and supporting cells (Figs. 3c, 3d).

It is noteworthy that mitochondria in cells of *B. nikolskii* olfactory epithelium were usually vacuolated to different degrees (Figs. 3e, 3f), which was indicative of their swelling, with a consequent loss of functional activity and eventual degradation. Confocal microscopy of cytochemically stained preparations confirmed that the amount of positively stained (functionally active) mitochondria in the olfactory epithelium was reduced (Figs. 3h, 3i). Analysis of Z-stack images showed that their numbers in *B. nikolskii* were 2-fold smaller than in *C. baicalensis* ( $p = 0.047$ ) and 1.7-fold smaller than in *C. inermis* ( $p=0.119$ ) (Fig. 4).

Structural disturbances were detected, not only in dendrites and perinuclear cytoplasmic regions of receptor neurons, but also in their axons. The axons of such neurons were usually dilated to different extents and could be easily distinguished from those of other, structurally normal olfactory cells. They were devoid of microfilaments and intact mitochondria, and the electron density of their axoplasm was lower than in the axons of viable neurons (Fig. 3g). Morphological analysis of cross sections of axon bundles showed that the proportion of degenerating olfactory neurons averaged 5-10%. This proportion apparently included neurons undergoing natural cell death, which is known to occur in the olfactory epithelium throughout the animal life span [16].

## DISCUSSION

Morphological studies on the olfactory rosettes of the three Baikal sculpin species were performed in order to reveal the cytological features characteristic of olfactory cells in the norm and discriminate them from the results of rearrangements caused by depressurization upon delivery to the surface. To this end, we used not only published data but also the criteria for evaluating the structure and functional status of receptor cells that were determined in our previous studies on another Baikal sculpin, *Cottocomephorus grewingkii* (Dybowski, 1874), at different stages of the life cycle [17,18], which appears to be methodologically valid. Thus, cytological features characteristic of the olfactory cells of *C. grewingkii*, which is not exposed to changes in hydrostatic pressure during the period of living in coastal waters, were taken as control.

The results of comparative morphological analysis showed that the olfactory receptor cells in *B. nikolskii* have a higher level of ultrastructural development compared to those in the two pelagic species. They contain a well-developed Golgi apparatus, an extensive network of ER canals and abundant ribosomes, both free and ER-bound. A probable explanation is that *B. nikolskii*, which has adopted a benthic mode of life, is exposed to higher sensory load compared to the pelagic species, because the benthic environ-

ment is more saturated with organic substances of different origin. It should be noted that, according to measurements of chemical oxygen demand (COD) by dichromate method [19], the contents of organic matter in Baikal waters are generally low. Thus, COD in the pelagic zone averages 3-4.5 mg O<sub>2</sub>/L [20]; however, this parameter markedly increases in the near-bottom water and especially in water from the upper layer of bottom sediments, reaching 8-10 and 20-30 mg O<sub>2</sub>/L, respectively. Moreover, calculations show that the rate of organic matter decomposition in the upper layer of bottom sediments reaches 2.5 g C<sub>org</sub>/m<sup>2</sup> per year [21], with this process being a constant source of olfactory signals.

It is noteworthy that both pelagic and benthic species included in the study had signs of destructive changes in cells of the olfactory epithelium, which included fragmentation and vacuolation of rough ER canals and structural disturbances in the axons and dendrites. No such ultrastructural alterations have been revealed in the Baikal sculpins that live in the coastal zone and are not exposed to abrupt changes in hydrostatic pressure [18]. Therefore, it appears that the above changes are the consequence of hypobaric hypoxia developing in deep-water fish upon delivery to the surface.

Another point of interest concerns the degree of preservation of mitochondria, which are especially vulnerable to oxygen deficiency [14]. Our data show that the morphological stability of mitochondria is higher in the pelagic species, *C. baicalensis* and *C. inermis*, and the same is true of their functional activity: as can be seen in Fig. 4, the proportion of mitochondria with an intact level of oxidative phosphorylation in these species is, on average, two-fold higher than in the deep-water benthic *B. nikolskii*.

Are there special mechanisms in the peripheral olfactory apparatus of the fish that can ensure the safety of cells and their organelles under extreme conditions of acute hypoxia? As noted above, foraging pelagic fish periodically ascend from depth to surface and then descend again, following the movements of their prey. In the course of evolution, such a regime of alternat-

ing exposure to high and low hydrostatic pressures could well provide for the development of relevant metabolic adaptations. Therefore, it appears that the good preservation of mitochondria in the olfactory receptor cells of pelagic fish could be due to the protective effect of hypoxic preconditioning, which has been recently revealed in experiments on mammals. It has been shown that animal exposure to interval hypobaric hypoxia leads to the activation of adaptive genetic mechanisms improving the tolerance of the brain to oxygen deficiency [22]. These mechanisms include the activation of early genes responsible for the production of antiapoptotic factors, cytoplasmic and mitochondrial antioxidants, metalloproteases, heat shock proteins and other agents protecting nerve cells from adverse effects under conditions of acute cerebral ischemia. These data concern terrestrial mammals, which have always lived in an atmospheric-pressure environment and could not develop any evolutionary adaptations to hypobaric hypoxia. Therefore, it may well be that fish species adapted to a wide range of hydrostatic pressures have effective mechanisms providing for a high tolerance to oxygen deficiency, and analysis of these mechanisms is of major theoretical and practical importance. In particular, studies on these fish can open an opportunity to identify new neuroprotector molecules improving the resistance of the brain to ischemic damage, which is known to be responsible for the development of many socially significant neurodegenerative disorders [23].

Another important cytological feature of olfactory neurons in deep-water fish species is that their dendrites contain a well-developed system of microtubules. In our previous studies, cytoskeletal elements in the olfactory receptor cells of coastal sculpins (*C. grewingkii*) were detected only in the spawning period, when these receptors were tuned to the perception of sex pheromones [17,18]. Moreover, we found that microtubules in the dendrites of fish chemoreceptor cells formed a distinct structural pattern after the fish were kept for a long time in clear water poor in organic substances [24]. This is evidence that well-organized cytoskeletal structures in the dendrites of receptor cells are essential for the maintenance of cell structural integrity under conditions of deficit in sensory signals.

Thus, the cytoskeleton of olfactory neurons acquires a highly structured pattern not only under high functional load in the spawning period but also in waters with low concentrations of dissolved substances. It also appears that the structural and functional characteristics of receptor neurons in the olfactory epithelium are not common but individual to each of them, because these characteristics depend not only on the initial, genetically determined sensitivity of receptors [25], but also on the effect of environmental factors on their expression [26,27] and the potential of these cells for subsequent stimulus-dependent differentiation [28,29].

## CONCLUSIONS

The structural and functional status of olfactory receptor cells in closely related deep-water sculpin species (Cottoidei) from Lake Baikal is largely determined by the hydrostatic pressure regime (depending on their mode of life) and chemical composition of the water. In pelagic species, the receptor cells are characterized by a low level of organelle development, and their structural integrity is maintained due to a well-organized system of cytoskeletal elements. The reduced functional status of receptor cells can be explained by the low contents of organic substances (potential olfactory stimuli) in the oligotrophic lake. In contrast, the olfactory neurons of benthic sculpins, which live in an environment enriched with various chemical substances, have a well-developed system of cell organelles, including an extensive network of endoplasmic reticulum canals and a high density of ribosomes. The results of cytochemical analysis of the peripheral olfactory apparatus in sculpins sampled from their natural environment provide evidence for a direct correlation between the level of receptor cell development and the overall intensity of olfactory signaling from the environment. Electron microscopic data and quantitative analysis for functionally active mitochondria in the olfactory epithelium of deep-water sculpins show that their exposure to hypobaric hypoxia during delivery to the surface leads to degenerative changes in the olfactory cells. Moreover, the extent of these changes depends on fish mode of life: it is markedly

greater in *B. nikolskii* (a typical Baikal low-mobile benthic fish) than in pelagic *C. baicalensis* and *C. inermis*, which are regularly exposed to changes in hydrostatic pressure during vertical foraging migrations. This is evidence that closely related fish species evolutionarily adapted to living under different hydrological conditions have different levels of tolerance to hypoxia. The cytological data obtained in this study are important for understanding basic mechanisms ensuring the reliable functioning of the olfactory apparatus under different environmental conditions. Moreover, they show that hydrobionts adapted to changes in hydrostatic pressure are promising as models for the search of neuroprotective agents that improve the tolerance of nerve cells to hypoxia and other extreme environmental factors.

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## REFERENCES

1. Coppola DM. Studies of olfactory system neural plasticity: the contribution of the unilateral naris occlusion technique. *Neural Plast.* 2012;2012:351752.
2. Ruan Y, Zheng, XY, Zhang HL, Zhu W, Zhu J. Olfactory dysfunctions in neurodegenerative disorders. *Neurosci Res.* 2012;90:1693-700.
3. Kasumyan AO. The Olfactory System in Fish: Structure, function, and role in behavior. *Ichthyol.* 2004;44(2):180-223.
4. Reuter K, Kapoor BG. *Fish Chemosenses.* Science Publishers, Inc. USA; 2005.
5. Hara TJ, Zielinski B. Olfaction. *Sensory Systems Neuroscience.* Fish Physiology Eds. Farrell AP, Brauner CJ. San Diego: CA. Academic Press. 2006;25:1-43.

6. Fishelson L, Golani D, Galil B, Goren M. Comparison of the nasal olfactory organs of various species of lizardfishes (Teleostei: Aulopiformes: Synodontidae) with additional remarks on the brain. *Int J Zool.* 2010;2010:807913.
7. Popper AN. Scanning electron microscopic studies of the sacculus and lagena in several deep-sea fishes. *Am J Anat.* 1980;157:115-36.
8. Marshall NJ. The lateral line system of three deep-sea fish. *J Fish Biol.* 1996;49:239-58.
9. Raymond EH, Widder EA. Behavioral responses of two deep-sea fish species to red, far-red, and white light. *Mar Ecol Prog Ser.* 2007;350:291-8.
10. Wagner HL. Sensory brain areas in three families of deep-sea fish (slickheads, eels and grenadiers): comparison of mesopelagic and demersal species. *Mar Biol.* 2002;141(5):807-17.
11. Herring PJ. *The Biology of the Deep Ocean.* Oxford: Oxford University Press; 2002.
12. Kirylchik SV, Slobodyanyuk SI, Belikov SI, Pavlova ME. Phylogenetic relationships among 16 species of sculpins Lake Baikal by analyzing the nucleotide sequence of the gene fragment in the mitochondrial DNA cytochrome. *Mol Biol.* 1995;29(4):817-25.
13. Sideleva VG. *Endemic fishes of Lake Baikal.* Leiden: Backhuys Pub; 2003.
14. Vettier A, Labbe C, Amerand A, Da Costa G, Le Rumeur E, Moisan C, Sebert P. Hydrostatic pressure effects on eel mitochondrial functioning and membrane fluidity. *Undersea Hyperb Med.* 2006;33(3):149-56.
15. Casillas E, Smith L, D'Aoust BG. The response of fish blood cells, particularly thrombocytes, to decompression. *Undersea Biomed Res.* 1976;3(3):273-81.
16. Graziadei GA, Graziadei PP. Neurogenesis and neuron regeneration in the olfactory system of mammals. II. Degeneration and reconstitution of the olfactory sensory neurons after axotomy. *J Neurocytol.* 1979;8(2):197-213.
17. Klimenkov IV. Ultrastructural rearrangement in receptor cells of olfactory analyzer in fishes at different stages of life cycle. [dissertation]. [Irkutsk]: Irkutsk State University. 1990. 155 p.
18. Kositsyn NS, Klimenkov IV. Transformation of elements of cytoskeleton in receptor cells of olfactory analyzer in fishes at different stages of life cycle. *Dokl Ross Akad Nauk.* 1994;336(2):261-3.
19. Skopintsev BA. Organic matter in natural waters. *Trudy GOIN.* In: Proceedings of GOIN. 1950;17(29):5.
20. Votincev KK, Meshcheryakova GI, Popovskaya GI. Organic matter turnover in the Lake Baikal. Novosibirsk: Nauka; 1975.
21. Votintsev KK. On the question to the modern sedimentation in Lake Baikal. *Reports of the Academy of Sciences.* 1967;174(2):419-22.
22. Rybnikova E, Glushchenko T, Tyulkova E, Baranova K, Samoilo M. Mild hypobaric hypoxia preconditioning up-regulates expression of transcription factors c-Fos and NGFI-A in rat neocortex and hippocampus. *Neurosci Res.* 2009;65:360-6.
23. Pluta R. From brain ischemia-reperfusion injury to possible sporadic Alzheimer's disease. *Curr Neurovasc Res.* 2004;1(5):44-153.
24. Klimenkov IV, Kositsyn NS. Morphological reorganizations of olfactory neurons at fishes after their periodic stimulation by sucrose. *The bulletin of Irkutsk State University.* 2008;2:33-6.
25. Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell.* 1991;65(1):175-87.
26. Wang HW, Wysocki CJ, Gold GH. Induction of olfactory receptor sensitivity in mice. *Science.* 1993;260:998-1000.
27. Harden MV, Newton LA, Lloyd RC, Whitlock KE. Olfactory imprinting is correlated with changes in gene expression in the olfactory epithelia of the zebrafish. *J Neurobiol.* 2006;66:1452-66.
28. Klimenkov IV, Kositsyn NS, Svinov MM. Common features of stimulus-dependent differentiation of olfactory receptor neurons and b cells of the immune system. *Dokl Ross Akad Nauk.* 2011;436(2):273-5.
29. Klimenkov IV, Kurylev AV, Kositsyn NS, Sudakov NP, Pastukhov MV, Nikiforov SB, Belykh EG, Byvaltsev VA. Olfactory receptor cells' dendritic neurosecretion phenomenon. *World Neurosurgery.* 2015;83(3):278-9.