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COMPARATIVE AND ONTOGENIC PHYSIOLOGY

Cellular Basis of Temperature Dependence of the Food-Procuring Activity of the Mollusc *Lymnaea stagnalis*

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Abstract—Neuronal correlates of temperature dependence of alimentary behavior were studied in experiments on molluscs. It was found that a decrease of temperature led to suppression of food-procuring activity of the animals: to a decrease of the consumed food amount and of the number of food holes on the substrate. These behavioral changes are associated with a fall of impulsation frequency of the motoneuron alimentary network and with decrease of efficiency of synaptic transmission within the limits of central generator of the *Lymnaea stagnalis* alimentary rhythm. It is suggested that change of character of intercellular interactions within the CNS limits underlies the temperature dependence of the mollusc food-procuring activity.

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Key words: neuron, synapse, central rhythm generator, invertebrates.

INTRODUCTION

Temperature is one of the basic abiotic factors in the living nature. The temperature dependence and/or sensitivity of the majority of biological reactions have cast no doubts as long as for several centuries of development of the natural science. Meanwhile, the character and the neuronal mechanisms of the temperature dependence of various forms of the behavioral activity have remained poorly studied, especially in the comparative-physiological aspect. The ectothermal organisms, i.e. the animals whose body temperature "follows" the environmental temperature provide the unique methodic possibility of study of the temperature factor action on the organism, including its nervous functions. Many of the poikilothermal invertebrates (worms, molluscs, crustaceans, and insects) are widely used as model neurobiological objects whose neuronal networks are studied sufficiently well [11].

Generation and subsequent maintenance of the periodic motor activity, for example, locomotion and respiration, is provided due to functioning of the central rhythm generators (CRG)—the peculiarly arranged neuronal networks from the CNS composition [2, 3]. The formation of the CRG structure represents the common principle of the brain organization both in invertebrates and in invertebrate animals [4].

In the fresh-water pulmonary mollusc *Lymnaea stagnalis*, the alimentary process occurs due to rhythmic movements of radula whose scarping movements on the alimentary substrate provide the food catching and its further translocation along the digestive tract. The whole cycle of the radula movement consists of three parts with approximately equal duration (about 3 s): the protraction (moving-out), the movement during the alimentary substrate (retraction), and swallowing (the propulsion of the alimentary particles into esophagus). Even in the case of use of isolated nervous

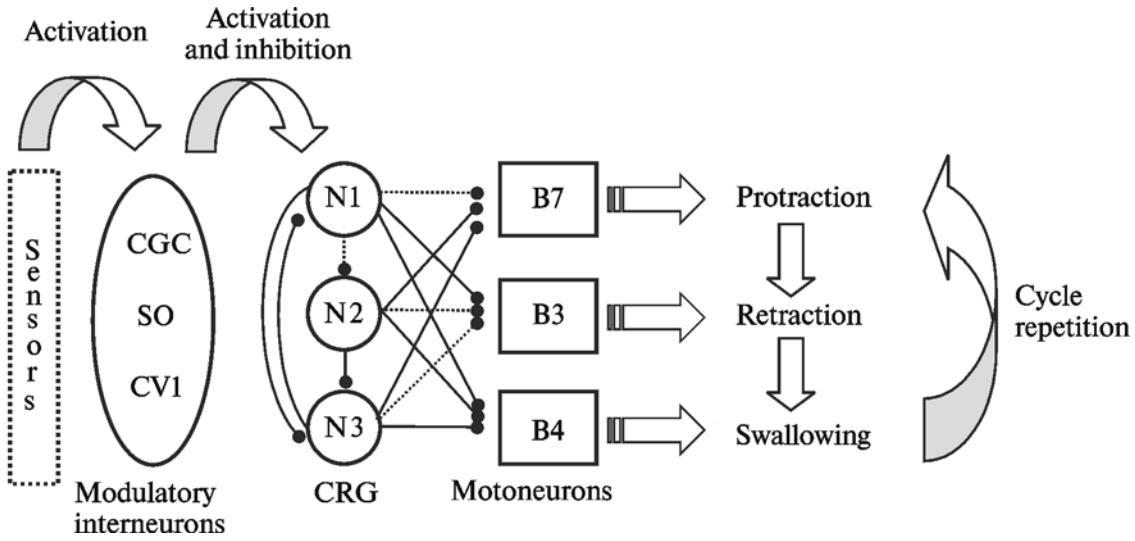

Fig. 1. Scheme of the neuronal network controlling the mollusc *Lymnaea stagnalis* food-procuring activity (from [6]), with changes and simplifications. *Solid lines*—inhibitory, *broken lines*—excitatory chemical synapses; the scheme does not indicate electriotonical connections between N1 and B7, N3 and B4, ad well as the character of interaction between the modulatory, sensory neurons and the central generator interneurons (N1—№3). The detailed description — see in the text.

systems, well seen is the periodic impulsation of neurons of the food-procuring network mediated these radula movements [5]. The electrical activity of practically any of the alimentary network neurons can be the source of information about frequency and duration of the alimentary rhythms in the CNS.

The rhythmic radula movements are controlled by the motoneuron network located in buccal ganglia (B1–B10) (Fig. 1). In turn, their activity is under the constant control of interneurons of the alimentation CRG. Three types of these neurons are identified: neurons 1 are responsible for the protraction phase, 2—for the retraction phase, and 3—for the swallowing phase. All of them form the chemical inhibitory and excitatory, and in some cases also electrotonic synapses with the corresponding motoneurons. The modulating elements are interneurons of the central ring of ganglia—the gigantic cells (CGC) and the cells of the 1st ventral cluster (CV1) of cerebral ganglia or interneurons of the buccal ganglia themselves (neurons of the slow oscillator, SO). Activation of the nutrition CRG results from the in-flow of signals from the periphery (from sensory neurons and sensory endings of the interneurons themselves of the alimentary network). As a rule, these are different chemical stimuli [6–9].

The goal of this work was to study the temperature dependence of the functioning of the central generator of the alimentary rhythm of the *Lymnaea stagnalis* nervous system as well as the temperature-mediated change of the mollusc food-procuring activity.

MATERIALS AND METHODS

The work is carried out on a representative of the fresh-water mollusc the pond snail *Lymnaea stagnalis* (L.). At laboratory, the animals were kept in aquarium at 24–26°C; each mollusc has no less than 1 l of water. The young leaves of the salad and dandelion were the food. Used in the experiments are adult individuals with the body mass of 3–4 g (the shell length of 25–35 mm). To study the food-procuring activity, the Petri dish with the mollusc submitted to the food deprivation for 24 h was added by the previously weighed plate cut out from a salad leaf or dandelion. After 2 h the plate was dried with a filter paper, weighed, and the amount of the consumed food was determined; the alimentary orifices were noted and a decrease of food per one

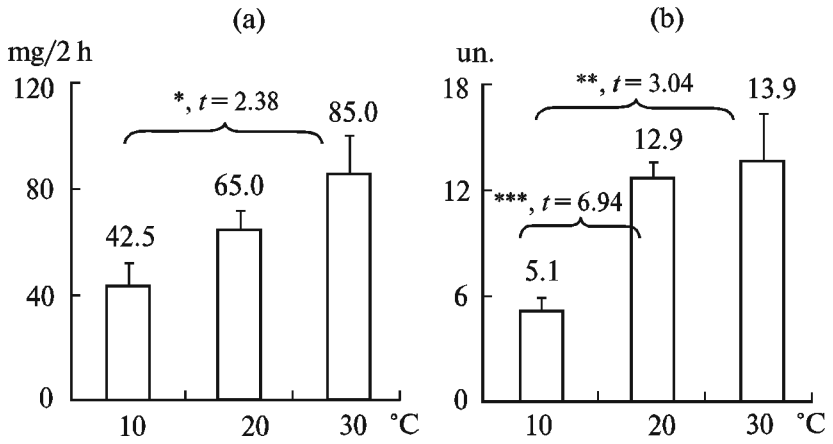

Fig. 2. Effect of temperature (°C) on the mollusc *Lymnaea stagnalis* alimentary behavior. (a) Amount of the consumed food (mg/2 h); (b) the number of the alimentary holes (units). The values of the studied parameters are presented (*digits above the columns*). The number of individuals (*n*) is 10 for each experimental series. Asterisks designate the statistically significant differences: *—$p < 0.05$, **—$p < 0.02$, ***—$p < 0.01$. The *t*-criterion value is indicated for the statistically significant differences.

orifice was calculated. The study of the food-procuring behavior was performed at temperatures of 10 ± 0.5, 20 ± 0.5, and 30 ± 0.5°C.

Electrophysiological experiments were performed using preparations of the isolated CNS. To soften perineural sheath and to facilitate penetration of microelectrodes into neurons, the preparations were previously treated with pronase solution (Protease E, type XIV, Sigma, USA) at the 1 mg/ml concentration prepared in normal saline for the *Lymnaea stagnalis* for 5 min at 20°C. The neuron electrical activity was recorded after 30 min of washing of the treated preparation with fresh saline. The intracellular recording of the neuron electrical parameters was performed using the Ag/AgCl electrodes. The micropipettes were filled with 2.5 M KCl solution (the microelectrode resistance was 10–40 MOhm). A chlorinated silver wire was used as the indifferent electrode. The amplified electrical signals were fixed on the type of an H327-3 recorder and were reflected in parallel on the screen of a C1-74 memorizing oscillograph.

For perfusion (0.1 ml/min) of preparations of the isolated nervous system, there was used the normal saline containing (mM): 44.0 NaCl, 1.7 KCl, 4.0 $CaCl_2$, 1.5 $MgCl_2 \cdot 6.0\ H_2O$, 10 HEPES; pH 7.5 ± 0.03.

The neurons were identified by the size, location within the CNS limits, the color, the electrophysiological characteristics of the action potential, the rest potential value, and the spontaneous activity pattern. The work is performed on the CNS cells involved in realization of the *Lymnaea stagnalis* food-procuring behavior: the motoneurons of the alimentary CRG B4 and B4-cluster [6, 8, 9].

The camera specially constructed on the basis of the Peletier semiconductor element provided a possibility of maintaining and change of the designed experimental temperatures with precision of ± 0.1°C at the diapason from 2 to 35°C.

The experimental data were treated with methods of variation statistics [10]. The number of observation (*n*) is indicated separately for each series of experiments. The data are presented as $x \pm S_x$. The statistical significance of the obtained data was evaluated by using Student's criterion. The results were considered statistically significant at the *p* level lower than 0.05.

RESULTS

Effect of temperature on the Lymnaea stagnalis food-procuring activity. The environmental temperature produces a marked change of parameters of the mollusc alimentary behavior (Fig. 2). Thus, the consumed food amount decreased 2 times in the

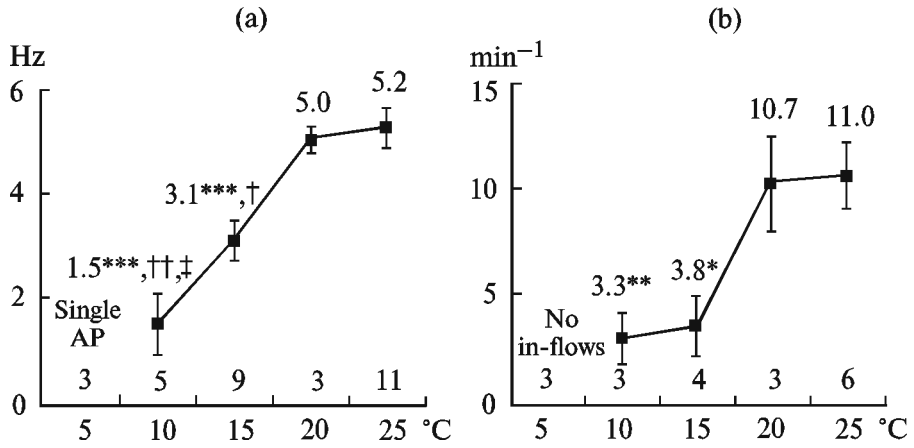

Fig. 3. Effect of temperature (°C) on electrical activity parameters of neurons (R/L B4, R/L B4-cluster) of the mollusc *Lymnaea stagnalis* alimentary network. (a) Frequency of the action potential generation (Hz), (b) the alimentary rhythm frequency (min^{-1}). The values of the studied parameters (*digits above the graph dots*) and the number (*n*) of the used nervous system preparations (*digits above the abscissa*) are presented for each experimental series. Asterisks designate the statistically significant differences: *—$p < 0.05$, **—$p < 0.02$, ***—$p < 0.01$ as compared with parameters at 25°C ; †—$p < 0.05$, ††—$p < 0.02$ as compared with parameters at 20°C ; ‡—$p < 0.02$ as compared with parameters at 15°C.

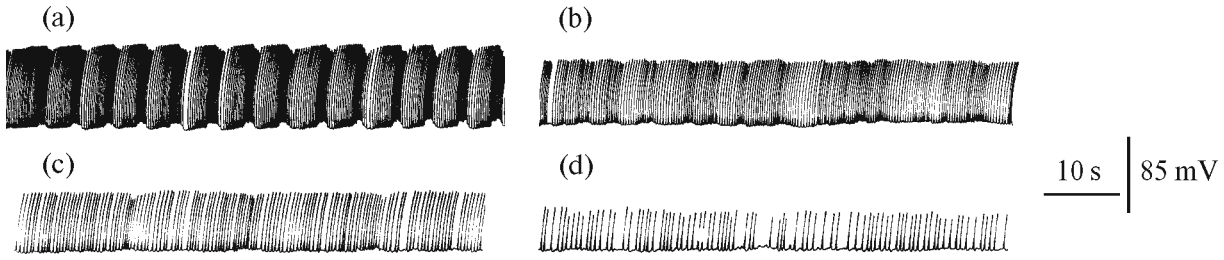

Fig. 4. Spontaneous electrical activity of the neuron of the LB4-cluster from the composition of the mollusc *Lymnaea stagnalis* alimentary network. Temperature (°C): (a) 25, (b) 20, (c) 15, (d) 10.

mollusc at 10°C as compared with that at the temperature of 30°C. In parallel, the number of alimentary holes at 10°C decreased as compared with that at the temperatures of 20 and at 30°C 2.5 and 2.7 times, respectively. It is also to be noted that with elevation of temperature the size of the alimentary holes increased and individual alimentary holes as if fused with each—the mollusc bites the "alimentary tracks" in the substrate. Nevertheless, no statistically significant differences in the food consumption per one alimentary hole was noticed at different temperatures: at 10 and 20°C 9.6 ± 2.62 and 5.0 ± 0.45 mg/hole, respectively ($p > 0.05$, $t = 1.74$), at 30°C 7.0 ± 0.91 mg/hole ($p < 0.05$, $t = 1.98$ as compared with 20°C); $n = 10$ for each experimental series.

Effect of temperature on the electrical activity of neurons of the Lymnaea stagnalis of the food-procuring network. Analysis of neurograms obtained at recording of the spontaneous electric activity of neurons R(L) of B4 cluster and cells R(L) B4 has shown that they preserved the ability to generate action potentials at all studied temperatures in the diapason of 5–25°C (Figs. 3a and 4). The maximal frequency of impulsation and the action potential amplitude are observed at 20–25°C. A temperature decrease to 15°C leads to a more than the 1.5-fold decrease of the cell spike activity as compared with that at 20°C under the background of the developing depolarization. The action potential amplitude decreases statistically significantly by amounting to 76.0 ± 4.45% of the value determined

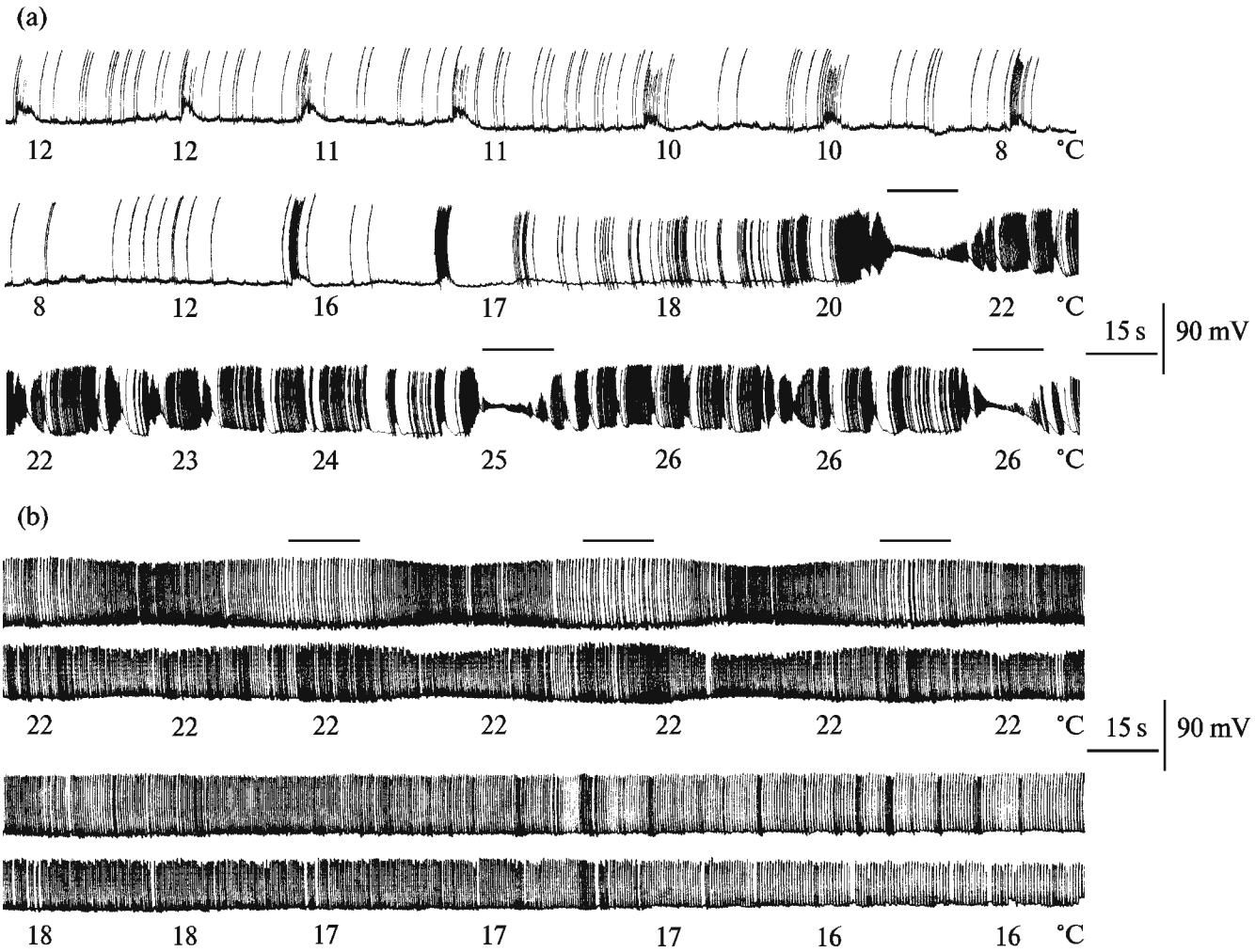

Fig. 5. The temperature dependence (°C, the values—under the activity recording) of the alimentary rhythm in the mollusc *Lymnaea stagnalis* nervous system. (a) Spontaneous electrical activity of the neuron of the RB4-cluster (*continuous recording*); (b) spontaneous electrical activity of neurons LB4 (*the upper band*) and RB4 (*the lower band*). Simultaneous recording of activity of two cells is presented. The time interval between two recordings is 1 min. The horizontal line indicates the long-period synaptic in-flows.

at 20–25°C ($n = 7$). A further temperature reduction to 10°C decreases frequency of the bursts even more—twice as compared with that noted for 15°C. The neurons are depolarized, the action potential amplitude reaches 55.3 ± 6.07% of the initial value at 20–25°C ($n = 5$). The temperature coefficients Q_{10} calculated for the impulsation frequency amount to 1.68 (the diapason of 20–15°C) and to 3.33 (the diapason of 20–10°C). At 5°C, only single, non regular action potentials are recorded, with their amplitude not differing statistically significantly from those observed at 10°C.

The alimentary rhythm in the Lymnaea nervous system also turned out to be submitted to the temperature modulation (Figs. 3b and 5a). At 20–25°C, on the membrane of the buccal neurons B4 and B4-cluster, periodic synaptic in-flows are recorded, the so-called alimentary rhythm with frequency of 10–11 cycles/min. A temperature decrease to 15°C leads to an almost 3-fold decrease of the alimentary rhythm frequency and to a decrease of the synaptic in-flow amplitude (to 20.03 ± 2.8%, $n = 4$). Simultaneously, duration of inter-burst intervals increases from 5.73 ± 1.57 at 20–25°C ($n = 6$) to 21.2 ± 3.67 at 15°C ($n = 4$). A further temperature decrease to 10°C does not lead

to statistically significant changes of the alimentary rhythm frequency and of duration (22.1 ± 4.37 s) of the inter-burst intervals (n = 4). It is also to note a more than 2.5-fold increase of duration of the synaptic in-flows to 4.7 ± 0.68 (n = 4), as compared with that observed at the temperature of 20–25°C (1.8 ± 0.12 s, n = 6). The temperature coefficients Q_{10} calculated for frequency of the synaptic in-flows to the motoneurons of the buccal ganglia amount to 2.89 (the diapason of 20–15°C) and to 3.29 (the diapason of 20–10°C). At 5°C, the pronounced alimentary rhythm practically disappears.

It is to be noted that necessary to note, that the reduction of efficiency of synaptic transmission at a decrease of temperature is also characteristic of the contacts formed most likely by modulator neurons of the Lymnaea alimentary network. Thus, in several preparations, there is well seen the disappearance of the long-period in-flow providing the counterphase activity of the neurons B4 of the right and left buccal ganglia at 15°C (Fig. 5b).

The observed changes of the spontaneous electrical activity of neurons R(L) of the B4-cluster and cells R(L) B4 were reversible. The return of the nervous system preparations to the initial temperature conditions restores the frequency characteristics of activity of these cells.

DISCUSSION

The temperature dependence of behavior activity is a sufficiently well-studied phenomenon. As a rule, a decrease of the environment temperature is associated with inhibition of various forms of behavior of animals including molluscs [11, 12]. Thus, a sharp inhibition of alimentary activity is detected under the long-lasting effect of low temperatures (5°C) in *Lymnaea truncatula* [13]. Zoological observations also indicate that under the natural conditions in winter, i.e., at low environmental temperatures, the fresh-water pulmonary molluscs are immersed into the silt on the reservoir bottom, where they are in the inactive state and remain in the torpid state until the beginning of spring [14].

The central generator of the alimentary rhythm belongs to the so-called network oscillator, i.e., the neurons forming it do not have their own pacemaker activity, while the total activity of the neuronal population is the consequence of specific organization of synaptic contacts within the limits of the network [15]. The fall of temperature is known to be accompanied by deceleration of impulsation in the functionally different neurons—motor, sensory, interneurons, modulatory, both in vertebrate [16] and in invertebrate animals [17]. We detected almost linear (the graphic flattens out in the diapason of 20–25°C) temperature dependence of the generation frequency of the action potential of the alimentary network motoneurons in the diapason of 5–25°C, which little differs these cells from other motor neurons in the *Lymnaea stagnalis* nervous system [18]. Somewhat different is the temperature dependence of the synaptic in-flow within the limits of alimentary network, which is indicated by more than 1.5-fold difference in the coefficient Q_{10} value within the diapason of 25–15°C as compared with the similar parameter for the impulsation frequency of nerve cells. In combination with disappearance of the long-period in-flows (Fig. 5b), this fact undoubtedly indicates the higher temperature sensitivity of the alimentary rhythm, i.e., of electrical activity of central neurons of the central alimentary network interneurons. A decrease of the synaptic in-flows is observed in parallel with a decrease of their amplitude and an increase of the duration, which indicates a change of characteristics of the synaptic transmission between the neurons of the alimentary network. Similar changes have also revealed at analysis of the temperature dependence of the respiratory rhythm in the Lymnaea CNS, whose arrest of functioning occurs already at 12.5°C [19].

In fresh-water pulmonary molluscs including *Lymnaea*, the whole specter of signal molecules is used as a transmitter of intercellular interactions within the limits of the alimentary network [20]. The key substances of them are glutamate providing realization of the retraction phase [21], nitrogen monoxide responsible for the alimentary rhythm activation [22], serotonin, dopamine, and some peptides with the expressed modulatory effect on neurons of the alimentary network [20, 23]. Earlier, the temperature dependence of the signal chemical transmission has been shown for the whole specter of synapses of different ergicity, including that for dopamine and FNRF-amidergic contacts [24, 25]. It is logical to suggest that other

synapses that use the above-mentioned substances as neurotransmitters also have a similar reaction to a change of temperature conditions.

Thus, the temperature dependence of the mollusc *Lymnaea stagnalis* alimentary activity is due to modulation of the synaptic transmission of the signal between the alimentary network neurons. Changes of characteristics of the intercellular communication process can have an adaptive significance for an increase of survival of individuals at fluctuations of the environmental temperature.

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