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INVESTIGATION OF CHANGES IN MEMBRANE POTENTIAL AND RHYTHMIC ACTIVITY OF THE RETZIUS NEURON UPON STIMULATION OF THE SENSORY P-NEURON

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Abstract

It was found that in "natural neural networks", ganglia of the nervous system of a leech, the frequency of rhythmic excitation (a series of nerve impulses, RE) of one neuron is modulated upon activation of other neurons. Changes in the electrophysiological characteristics of the leech Retzius cell in response to electrical stimulation of one of the sensory neurons (P-cells) were revealed. Registration of changes in the membrane potential of neurons, as well as electrical stimulation of the P-cell was carried out using microelectrodes introduced into the cells. It was found that during electrical stimulation, P-cells increase the frequency of spontaneous RE of Retzius cells, the membrane potential of P-cells increases, but Retzius cells do not change. With an increase in the duration of stimulation, the RE frequency increases in both the P-cell and the Retzius cell. It has been found that RE Retzius cells, upon stimulation of P-cells, arise against the background of RE of the Retzius cell. Thus, during RE of sensory neurons and synaptic transmission to the Retzius cell, RE frequency modulation occurs. According to the authors, changes in the frequency of spontaneous RE of the Retzius neuron in the "natural neural network" are associated not only with a change in the RE frequency during excitation along nerve fibers from skin receptors, but also with the transformation of RE both between cells of one ganglion and between cells in different ganglia leech nerve chain

Keywords

"Natural neural networks", leech, ganglion, action potential, rhythmic exitation

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Introduction. The realization of the functions of the nerve cell is associated with rhythmic changes in the membrane potential (action potential, AP), namely, the frequency of repetition and duration of the procedure [1–4]. It is obvious that the formation of interactions between individual neurons in the network play a major role change in the electrical properties (the level of membrane potential, rhythm AP, trace phenomena, etc.), so the physical and chemical state neurolemma, intracellular organelles and cytoplasm [5–10]. This problem is not only fundamental, but also of great practical importance, since it is disorders of the regulation of membrane and subcellular processes that cause pathology both at the level of an individual neuron and neuronal networks [11–13]. For example, the Retzius cell (R-cell) is able to "analyze" and transmit RE to several motor neurons, so that the animal makes complex movements, and in the case of thermal stimulation, food reflexes are triggered [10–12]. It has been proved that there are no insertions neurons between some sensory and motor neurons, i.e., the excitation is directly transmitted to the motor neuron and causes a reflex response [2–4]. The development of bioelectronics interfaces is an urgent task for the study of neuronal networks both for fundamental areas of science and in biomedicine. To develop a neural network model that can be controlled using interfaces, we proposed using leech ganglia and identified changes in the membrane potential, AP amplitude, and frequency of spontaneous rhythmic excitation of Retzius neurons during thermal stimulation (TS) of the animal's skin [2–4] Note that similar changes in the frequency of spontaneous RA in skin TS are also observed for interneurons with neuromodulator function (L-cells). These neurons are involved in regulating a number of leech motor reflexes, including contractions of the body wall. It is possible that in TS, an increase in the frequency of spontaneous RE of Rz neurons is accompanied by activation of L-neurons. All this indicates that in order to form the final model of a neural network, it is necessary to take into account specific electrical interactions between individual neurons [13–15].

The purpose of this work was to study the process of transformation of rhythmic activity between individual neurons of the leech ganglion.

Methods. The object of the study was identified leech segmental ganglia (*Hirudo medicinalis*) with afferent fibers extending from them, innervating areas of the animal's skin. Before the experiment, the animal was anesthetized with a 10 % ethanol solution [2, 3]. To isolate the ganglia, the leech was opened from the abdominal side and the selected ganglion was cleared of the vascular membrane. During the experiment, the ganglion was in a medium containing: 115 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5.4 mM

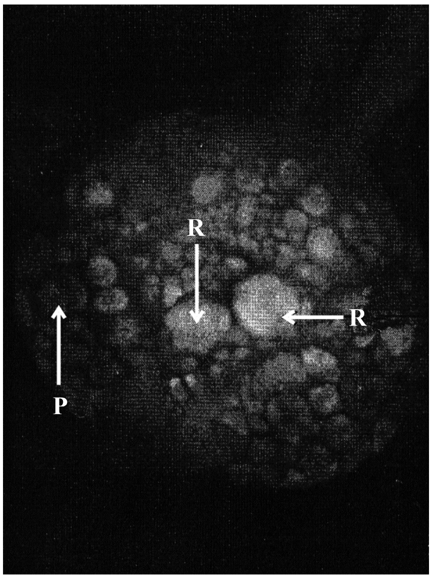

Fig. 1. Image of the segmental ganglion of a medicinal leech. The letters indicate R- (R) and P- (P) cells

Tris (pH 7.4). Neurons were visualized by inserting a carbocyanin probe DiI (Molecular Samples, Junction City, OR) dissolved in dimethylsulfoxide or ethanol (1–10 mg/ml) into the cell, and fluorescence was recorded using a confocal microscope (Biorad 1000/Nicon) (Fig. 1).

Registration of the membrane potential (MP) and action potential (AP) of Rz neurons was performed using microelectrode technology [2, 3]. The introduction of the microelectrode was carried out under visual control. The recording microelectrode was filled with 4M potassium acetate or 3M KCl. The microelectrode and comparison electrode (Ag–AgCl) were connected to an amplifier (Axoclamp-2A; Axon Instruments). Changes in MP and PD were analyzed using the Axon Instruments computer program [8, 12].

GraphPad Prism, version 8.02 (GraphPad Software, La Jolla California USA) was used for statistical analysis of the results. The study used 12 animals. All data were normally distributed (according to the generalized d'Agostino — Pearson normality test, $p < 0.05$).

Results and discussion. In connection with the assumption that Retzius cell RE can change due to activation of other ganglion neurons (in particular sensory neurons), changes in the membrane potential and frequency of Retzius cell AP in response to electrical stimulation of one of the sensory neurons — the P-cell were investigated. It was found that when the "silent" P-cell is stimulated, RE occurs both in the P-cell itself and in the Retzius cell (Fig. 2 *a*). At the same time, the membrane potential of the P cell and Retzius cell did not change, and the duration and amplitude of AP was greater in the P cell than in the Retzius cell. It was found that the duration of Retzius cell AP depends on the duration of P-cell AP (Fig. 2 *b*): as the duration of P-cell electrostimulation increased, the duration of Retzius cell RE increased, and the number of Retzius cell AP correlates with the number of P-cell AP. It was found that Retzius cell RE occurs against the background of the Retzius cell's own RA when P-cell is electro stimulated (Fig. 2 *c*).

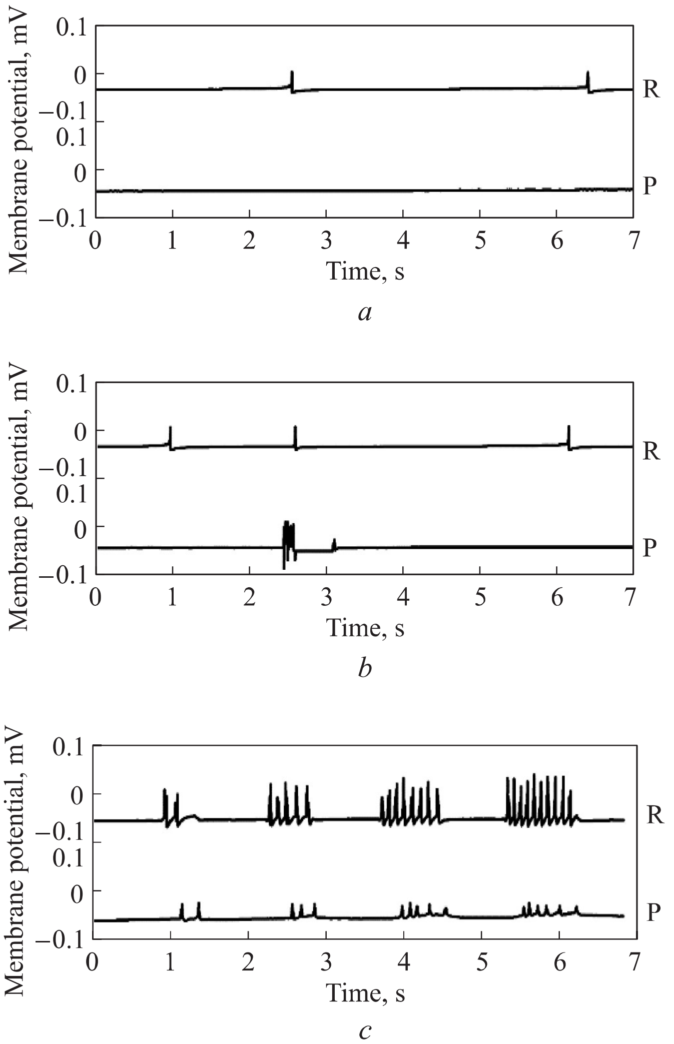

Fig. 2. Change of membrane potential and frequency of RE series of Retzius cells during electrical stimulation of P-cells; *a* rhythmic excitation of Retzius cells; *b* rhythmic excitation of the Retzius cell upon stimulation of the P-cell; *c* changes in the number of PDs in Retzius cells depending on the number of PDs in P-cells

Thus, the increase in the frequency of spontaneous RE of the Rz neuron is associated not only with the activation of skin cell receptors and, accordingly, additional modulation of RE in nerve fibers directly in contact with the Rz neuron, but also with the inclusion of additional (intermediate) intraneuronal connections, for example, between pressure-sensitive P-cells and the Rz neuron. It is obvious that in the leech's neural network, the Retzius cell compares the RE of sensory neurons with the RE of the Retzius cell [16, 17–19].

It was found that even before the generation of AP, R-cells are preceded by characteristic regular changes in the membrane potential (MP) (see Fig. 2 *b*). In this regard, we investigated the relationship between P- and R-cells, both in the formation of AP and duration of generation of the “pack” of RE. In these experiments, the following parameters were controlled: the number of APs and duration of generation of the “pack” of RE, as well as the dependence of the R-cell’s AP and “pack” of RE generation time on the amplitude and duration of P-cell stimulation. It was found that when the duration of electrostimulation in the P-cell increases, both the interval between the AP of the P-cell and the AP of the R-cell decreases. In the next series of experiments, it was found that the number of AP R-cell correlated with the number of P-cell AP, as with increasing the duration of the stimulating pulse (Fig. 2 and 3), and the amplitude of the stimulating pulse. It was found that the duration of the Retzius cell RE depends on the duration of the P-cell RE: as the P-cell stimulation time increases, the duration of the Retzius cell RE response increases [4, 5].

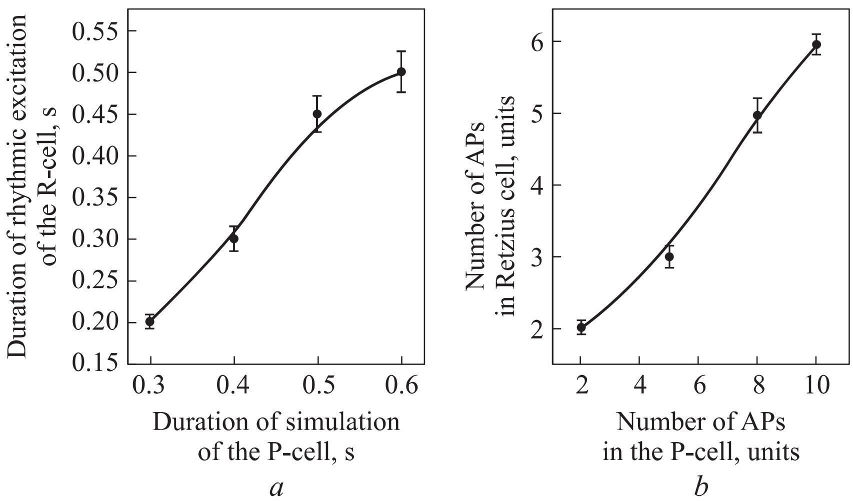

Fig. 3. The relationship between the rhythmic activity of P- and Retzius cells in the segmental ganglia of the medicinal leech; *a* the dependence of the duration of rhythmic excitation of the Retzius cell on the duration of stimulation of the P-cell; *b* dependence of the number of APs in the Retzius cell on the number of APs in the P-cells

So, the transformation of RE in the synapse from P- to R-cell and the generation of AP depend on the type of RE P-cell, and the generation of the first in this pack of AP is faster with an increase in the number of AP P-cells. Similar effects were found in the registration of R-cell AP, although no correlation was found between the number of R-cell AP and R-cells. Thus, changes in the frequency of spontaneous RE Rz neuron in various ganglia of the nervous chain in skin TS can be associated not only with the activation of the entire conducting system (nerve fibers directly associated with receptors in the skin), but also other cells (L-cells) or "silent" neurons (P-cells) [3, 11, 17–19].

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