
EXPERIMENTAL BIOLOGY

Changes in the Frequency of Rhythmic Excitation of Retzius Cells during Thermal Stimulation of Leech Skin

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Thermal stimulation of various parts of the skin in *Hirudo medicinalis* increases the frequency of spontaneous rhythmic excitation of Retzius neurons in leech ganglia. It was shown that the frequency of spontaneous rhythmic excitation of Retzius cells in the segmental ganglion increases only in response to thermal stimulation and returns to initial values upon cooling. This effect was also detected in neurons that are not directly connected by nerve fibers with the particular skin area. Changes in the frequency of spontaneous rhythmic excitation of Retzius cells in the segmental ganglion were observed during thermal stimulation of not only leech body, but also of the head and caudal suckers. These changes in spontaneous rhythmic excitation of Retzius cells in the segmental ganglion during thermal stimulation were observed in *Hirudo medicinalis*, but not in *Macrobdella decora*.

Key Words: *Hirudo medicinalis*; *Macrobdella decora*; rhythmic excitation; thermoreception

Thermoreception in leeches is determined by a complex reflex action due to activation of several neurons (receptor, motor, trigger and interneurons) in the ganglion [4]. One of these neurons is Retzius neuron (Rz-neuron), a trigger neuron that perform processing and integration of the frequency of spontaneous rhythmic excitation (SRE) of sensory neurons and transmit the signal to motor neurons [7]. Moreover, Rz-neurons produce and release serotonin that plays an important role in the regulation of SRE [7].

In this work, we studied changes in membrane potential, amplitude of action potential, and SRE frequency of Rz-neurons of segmental and subesophageal ganglia *Hirudo medicinalis* and *Macrobdella decora* leeches during thermal stimulation (TS) of skin receptors.

MATERIALS AND METHODS

Identified neurons in the ganglia with afferent fibers, innervating the skin of the head, body or tail areas of *Hirudo medicinalis* and *Macrobdella decora* were used as the experimental objects. TS of the skin receptors was performed by changing temperature of the fluid in a silicone tube contacting with animal skin [6]. The isolated ganglia were placed in a chamber that allows varying the temperature of leech skin and parallel recording of changes in membrane potential or fluorescence of an individual neuron in the ganglion.

During the experiment, the ganglion was placed in a medium containing (in mM): 115 NaCl, 4 KCl, 1.8 CaCl₂, 1 MgCl₂, and 5.4 Tris (pH 7.4). The neurons were visualized by staining with a membrane dye DiI carbocyanine probe (Molecular Probes). The fluorescence was recorded using a confocal microscope (Biorad 1000/Nicon). Rz-neurons of the 5-th and 6-th

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ganglia associated with the leech reproductive system were not used [6].

The membrane potential and action potential of Rz-neurons were recorded using microelectrode techniques [1]. The recording microelectrode was filled with 4 M potassium acetate or 3 M KCl (resistance 20–40 m Ω).

Statistical analysis of the results was performed using a demo version of GraphPad Prism 8.02 (GraphPad Software). All data were normally distributed (according to D'Agostino—Pearson omnibus normality test, $p < 0.05$). During the study, 25 animals of each species were used. Significance of differences between SRE value of the before and after TS was evaluated using the Kruskal—Wallis test followed by Dunn multiple comparison Post hoc tests ($p < 0.05$). The mean SRE frequency before, during, and after TS was calculated for each period of the experiment (about 200 sec).

RESULTS

The SRE frequency of Rz-neurons from the segmental leech ganglion at rest was 0.26 ± 0.02 Hz. During skin TS (temperature change from 20° to 35°C), the SRE frequency increases to 0.6 Hz over the first 1.5–2.5 min (Fig. 1, *b*, *e*); in parallel, depolarization of the neuronal membrane was observed. In 5 min after the start of stimulation, SRE frequency decreased to 0.5 Hz, which was obviously a result of excitation threshold elevation (Table 1). After cooling, hyperpolarization of the membrane (~ 5 mV) and a decrease in SRE frequency to 0.35–0.40 Hz were observed. It can be hypothesized that after reducing skin temperature, action potentials of neighboring neurons or neurons of

other ganglia forming the nerve cord increase outward potassium current in Rz-neurons, thus reducing SRE frequency. Activation of the sodium pump can also contribute to neuronal membrane hyperpolarization during generation of high frequency SRE.

Thus, the increase of SRE frequency of Rz-neurons in the segmental ganglion of leeches is observed only in response to temperature rise, while temperature decrease leads to restoration of the initial SRE frequency. This effect was also detected in Rz-neurons that are not directly connected by nerve fibers with this skin area. This fact demonstrates the possibility of modulating SRE frequency of Rz-neuron of the segmental ganglion during activation of other neurons [2].

Similar changes of the neurons membrane potential during TS were observed in subesophageal ganglia. Unlike the segmental ganglia, SRE frequency of Rz-neurons of the subesophageal ganglion during TS increased in 20–30 sec and did not recover even after the end of TS (Fig. 1, *a*, *d*). Then, the SRE frequency decreased almost to the control level, but membrane potential of the neuron remained high (depolarization) even after the temperature was reduced. The increase in SRE frequency in response to decreasing skin temperature suggests that Rz-neurons of the subesophageal ganglion respond to temperature changes, rather than its increase. Thus, changes in SRE frequency of the segmental and subesophageal ganglia neurons in response to TS of skin receptors are different, and this fact determines the behavior of the animal.

During TS, SRE frequency of Rz-neurons changed not only in the body region, but also in the head and caudal suckers (Table 2). TS of head sucker receptors, increased the Rz-neurons SRE frequency of both the

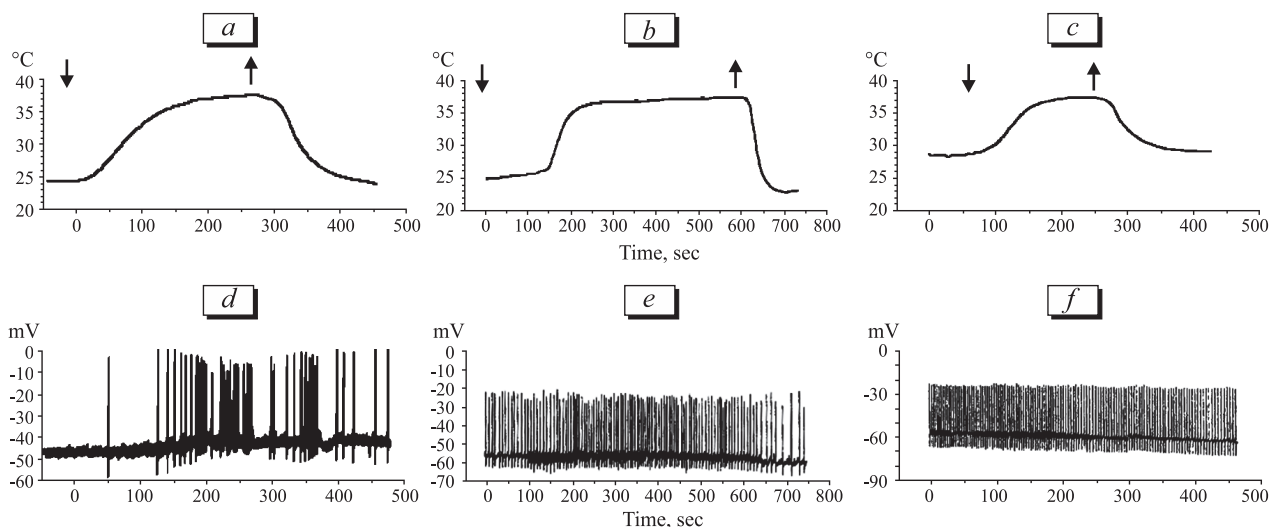


Fig 1. Typical kinetics curves of temperature changes (*a-c*) and amplitude of membrane potential (*d-f*) of Rz-neurons of the segmental (*a*, *d*) and subesophageal ganglion (*b*, *e*) of *Hirudo medicinalis* and segmental ganglion of *Macrobdella decora* (*c*, *f*) during thermal stimulation of the skin.

TABLE 1. Frequency of SRE in Rz-neurons during Thermal Stimulation of Leech Skin

Object	Rest	Thermal stimulation (200 sec)	Recovery (100 sec)
<i>Hirudo medicinalis</i>			
Segmental ganglion	0.26±0.02	0.61±0.03*	0.50±0.01
Subesophageal ganglion	0.28±0.05	0.36±0.08*	0.31±0.05
<i>Macrobdella decora</i>			
Segmental ganglion	0.31±0.13	0.29±0.23	0.33±0.08

Note. * $p=0.05$ in comparison with rest.

TABLE 2. Frequency of SRE of Rz-neurons in *Hirudo medicinalis* during Thermal Stimulation of the Skin

Neuron	Change in SRE
Head mesomeres	
Rz-neurons of the subesophageal ganglion	3.07±0.70*
Rz-neurons segmental ganglion	2.12±0.25*
Body	
Rz-neurons of the subesophageal ganglion	3.41±0.58*
Rz-neurons segmental ganglion	2.28±0.53*
Caudal mesomeres	
Rz-neurons segmental ganglion	3.40±0.63*

Note. * $p=0.05$ in comparison with the control. The data are standardized to the control (SRE frequency of the same neuron without thermal stimulation).

subesophageal and the following segmental ganglion in the leech nerve cord, but the changes in the subesophageal ganglion were more pronounced (~3.1 times from control) than in the segmental ganglion (~2.2 times). TS of the leech body also increased the SRE frequency in the subesophageal and segmental ganglia by 3.5 and ~2.3 times, respectively. These changes in rhythmic activity can be explained by the fact that axons of Rz-neurons transmit excitation to Rz-neuron of not neighboring, but next segmental ganglion.

It is known that each P-neuron, like N- and T-neurons, innervates a specific region of leech segment and projects its axons into the adjacent ganglia [1,3,7]. It is likely that the sensory neurons responsible for thermoreception also have their processes in the adjacent ganglion and excitation is transmitted to the adjacent ganglion by sensory neurons. It is important that during stimulation of the caudal sucker ganglion, changes in SRE of Rz-neurons in the segmental gan-

glion following the subesophageal were recorded. The amplitude of these changes was the same as in Rz-neurons directly associated with the innervated region.

We should emphasize that TS of the skin of *Macrobdella decora* was not followed by changes in SRE of Rz-neurons of the segmental ganglion (Fig. 1, c, f). It is known that, unlike *Hirudo medicinalis*, *Macrobdella decora* does not feed on warm-blooded hosts and TS is not accompanied with complex reflex response.

Thus, stimulation of skin thermoreceptors in leeches induces changes in SRE frequency of neurons. This response of Rz-neurons is associated with the transmission of excitation from the sensory neuron to Rz-neuron through synaptic contacts [1,3,5]. Moreover, the excitation from the sensory neuron is transmitted to Rz-neurons of the neighboring ganglia, thus introducing additional modulation of the signal. The response of Rz-neurons to temperature changes of leech skin differs in the body and head mesomeres, which is associated with the behavior of the animal (feeding, orientation, search for the victim). Therefore, different sections of the leech nervous system are characterized by different sensitivity to external signals. The changes in activity of Rz-neuron lead to excitation of motoneurons and, as a result, to the stimulation of muscle contraction and complex behavioral movements of the animal.

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REFERENCES

1. Baca SM, Marin-Burgin A, Wagenaar DA, Kristan WB Jr. Widespread inhibition proportional to excitation controls the gain of a leech behavioral circuit. *Neuron*. 2008;57(2):276-289.
2. Baljon PL, Wagenaar DA. Responses to conflicting stimuli in a simple stimulus-response pathway. *J. Neurosci*. 2015;35(6):2398-2406.
3. Burgin AM, Szczupak L. Network interactions among sensory neurons in the leech. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol*. 2003;189(1):59-67.
4. Groome JR, Vaughan DK, Lent CM. Ingestive sensory inputs excite serotonin effector neurones and promote serotonin depletion from the leech central nervous system and periphery. *J. Exp. Biol*. 1995;198(Pt 6):1233-1242.
5. Harley CM, Cienfuegos J, Wagenaar DA. Developmentally regulated multisensory integration for prey localization in the medicinal leech. *J. Exp. Biol*. 2011;214(Pt 22):3801-3807.
6. Maximov GV, Turovetskii VB, Chatterdji S, Andreev AI, Mironova YE, Brindikova TA, Rubin AB. Role of membrane-bound Ca²⁺ in regulating relationships between neuron and neuroglia during rhythmic excitation. *Biophysics*. 2000;45(3):529-533.
7. Velázquez-Ulloa N, Blackshaw SE, Szczupak L, Trueta C, García E, De-Miguel FF. Convergence of mechanosensory inputs onto neuromodulatory serotonergic neurons in the leech. *J. Neurobiol*. 2003;54(4):604-617.