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Serotonin Application Effects on Electrical Characteristics of the Premotor Interneurons in Intact and Trained Snails

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Abstract Experimentally, it was shown that the action potential threshold in neurons of the trained snails increases in response to serotonin (5-HT) application, in contrast to intact snails. This result demonstrates a decrease of excitability of the premotor interneurons in response to extracellular 5-HT after the training.

Keywords Serotonin · Identified neurons · Membrane and threshold potentials · Learning · Snail

1 Introduction

There are a large number of studies demonstrating the necessity of serotonin (5-HT) to develop conditioned defensive reflexes [1–5]. These results formed the basis for using application of 5-HT in the bathing solution as a reinforcing stimulus for the cellular analogues of learning [6–8]. A question arises, what are the mechanisms of 5-HT effect on the elements of the nervous system, allowing it to serve as a basic mediator for the defensive behavior in mollusks. Therefore, in the present work, we studied the excitability changes in the premotor withdrawal interneurons under the application of 5-HT in the isolated nervous system preparations made from intact and trained snails.

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2 Methods

The terrestrial snails *Helix lucorum*, the nervous system of which has been well described, were used for the experiments. Before the experiments, the animals had been in the active state for at least 2 weeks. The snails were trained to execute the defensive reflex on tapping on the shell [5]. Tapping on the shell (2 times) was used as a conditioned stimulus, which did not produce any defensive reaction in a snail under normal conditions. As an unconditioned stimulus, a puff of air into the lung cavity orifice (pneumostome) was used, which produced the defensive reaction of pneumostome closure in animals. The combinations of the stimuli were presented with a random interval that ranged from 2 to 4 min (to prevent the elaboration of a conditioned reflex to time). The defensive reflex was being elaborated over a 3-day period as a result of presenting 150 pairs of the conditioned and unconditioned stimuli. The electrical characteristics of the withdrawal interneurons of the snail's pneumostome closure reflex were analyzed. The recordings of the electrical characteristics were carried out on the next day after the training. The measurements were conducted using intracellular glass microelectrodes. The changes of the membrane (Vm) and threshold (Vt) potentials of the premotor interneurons in response to application of 5-HT (at a concentration of 1 mmol/l) into the solution bathing the nerve system of intact and trained snails were studied.

The results are reported as mean ± SEM. The unpaired Student's *t* test and non-parametric Mann–Whitney test were used for comparison between the two groups.

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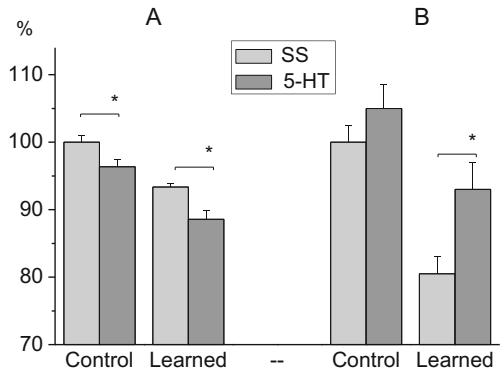

Fig. 1 Value of the resting membrane potential in percents (**a**) and the threshold potential (**b**) of the premotor interneurons LPa3, RPa3, LPa2, and RPa2 in the snails before (SS) and after the (5-HT) application of serotonin in the bathing solution preparation. Control—naive snails. Learned—trained snails. *The reliable difference ($p < 0.001$) versus Vm and Vt before application of serotonin

3 Results and Discussion

It was found that application of 5-HT causes a reliable decrease of Vm of the premotor interneurons of both naive (by 4 mV) and trained (by 3 mV) snails. In response to the application of 5-HT, a reliable increase of Vt of these neurons in the trained snails was as well found, in contrast to the naive animals, by 3 mV (Fig. 1). Thus, it was shown that the action potential threshold in the trained snails in response to the application of 5-HT increases, in contrast to the naive. This result demonstrates a decrease of excitability of the premotor interneurons in response to the extracellular 5-HT after the training.

The increase of neuron excitability under the action of serotonin was noted by a number of authors [9–12]. Jin with co-authors have shown that 5-HT increases the peak amplitude of the complex excitatory postsynaptic potential caused by the light enhances the internal excitability and the spike activity of the identified type Ie(A) interneurons of the *Hermissenda* mollusk [13]. We have also found that the application of 5-HT causes a decrease of Vm in the premotor interneurons of naive snails, which demonstrates the increase in the excitability of these neurons. From the works of Pivovarov A.S. and the colleagues, it follows that this may be due to the activation of serotonin receptors of the first type [14, 15]. However, the analysis of the excitability of these trained snails' interneurons showed that application of 5-HT to the preparation caused an increase in their Vt, while no changes were revealed in the Vt of the naive snails. This means that the appearance of extracellular 5-HT, which may be released, for example, from modulatory 5-HT-containing neurons [10], leads to a decrease in excitability of the premotor interneurons, increased after the training procedures. This fact can be of importance when analyzing plastic changes in the nervous system during learning, since the decrease of Vm and Vt while learning was shown [16, 17].

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