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Serotonin Modulation of Premotor Interneuron Excitability in the Snail during Associative Learning

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Abstract It is shown that after the elaboration of a conditioned reflex in snails, a reliable decrease can be observed in the membrane potential (Vm) of the premotor interneurons at 4 mV, daily injection of serotonin (5-HT) causes a decrease in Vm at 4.5 mV, the same change is observed for Vm in the snails trained after the injection of 5-HT. A single injection of 5-HT causes a depolarization shift of Vm at 5 mV. After the initial stage of training (10–12 pairs) the snails, injected by 5-HT, there is a depolarization at 4.5 mV.

Keywords Serotonin · Identified neurons · Membrane potential · Learning · Snail

1 Introduction

It is known that serotonin (5-HT) is an essential neurotransmitter of defensive behavior in mollusks; therefore, the role of the serotoninergic system in elaboration of defensive conditioned reflexes in mollusks is difficult to overestimate [1–4]. The serotonergic transmission from the modulatory neurons to the premotor interneurons is shown, including the release of 5-HT, from the modulatory neurons into the extracellular space [1, 5]. These results formed the basis for using the 5-HT application into the bathing solution as a reinforcing stimulus for elaborating the cellular analogs of learning [6–9]. Therefore, in this work, we tried to answer the question on what kind of cellular action mechanisms of 5-HT on the elements of the nervous system allows it to serve as a basic mediator for the defensive behavior of the mollusks. To solve this problem, we studied the effect of 5-HT injection in the membrane potential (Vm) of the premotor interneurons of trained and not trained snails.

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.

We have performed the following series of the experiments:

Experiment 1: Defensive reflex conditioning (CR) of food aversion. The CR of food aversion was elaborated in naive snails ($n = 10$) and in daily saline (SS) injected snails ($n = 10$). As a conditioned stimulus, a piece of cucumber was offered; as an unconditional stimulus an electric current of 1 mA value was used, which was presented at the time of the first chewing movements. The reflex was considered as elaborated after the snail avoided food 10 times in a row or, when being touched, showed a defensive response, without waiting for reinforcements [10].

Experiment 2: Effects of the daily injections of 5-HT on CR and Vm of the premotor interneuron. 5-HT was

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injected daily 1 h before the training session ($n = 10$) at a dose of 10 mg/kg. 5-HT was solved in 0.1 ml SS; in addition, as an antioxidant to the solution, ascorbic acid was added to achieve a concentration of 0.1 %. As a passive control, the injection of SS ($n = 5$) was performed for 4 days without training. As an active control, the injection of 5-HT ($n = 10$) was performed for 4 days without training.

Experiment 3: Effects of a single injection of 5-HT on the initial phase of training and Vm of the premotor interneuron. In a separate series of the experiments, we simulated the initial phase of learning. Earlier, we had shown that for full development of CR, 60–80 combinations of conditioned and unconditioned stimuli are required [10]. Therefore, based on the obtained results, we proposed 12 pairs of conditioned and unconditioned stimuli as the initial phases of training, which were applied during 1 day ($n = 10$). Single 5-HT had been injected 1 h before the training. As an active control, a group of snails was injected by single 5-HT, which did not receive training ($n = 5$).

Vm of the premotor interneurons was recorded using intracellular glass microelectrodes.

The results are reported as mean ± SEM. The unpaired Student *t* test and non-parametric Mann–Whitney test were used for comparison between the two groups.

3 Results and Discussion

3.1 Experiment 1

Conditioned reflex of food aversion was elaborated over 60–80 pairs of conditional and unconditional stimuli during 4 days, and a daily injection of SS did not change the rate of CR elaboration. After CR elaboration, the same (reliable) decrease in Vm of the premotor interneurons of 4 mV was observed both in the naive snails and snails injected by SS.

3.2 Experiment 2

It is shown that a daily injection of 5-HT before the training session accelerated the CR elaboration. It was found that daily injection of 5-HT during 4 days without training causes a decrease in Vm of 4.5 mV, and the same change is observed for Vm in the snails trained after the daily injection of 5-HT (Fig. 1). Any Vm changes are not observed in snails injected by SS for 4 days without training.

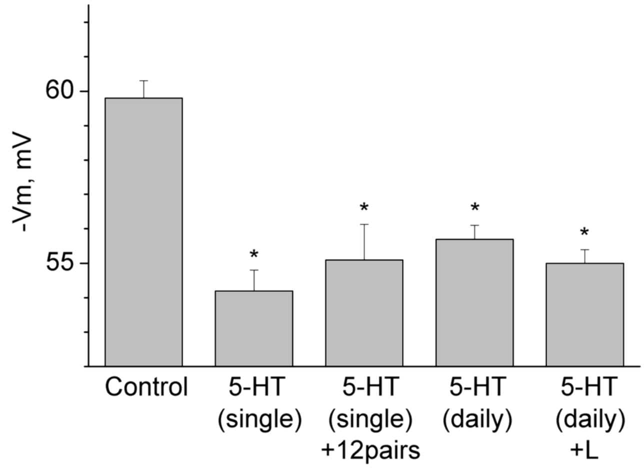

Fig. 1 The values of the resting membrane potential of the premotor interneurons LPa3, RPa3, LPa2, and RPa2 of the snails after various influences. 5-HT (single)—the snails after a single injection of 5-HT; 5-HT (single + 12 pairs)—the snails which received 12 pairs of conditioned and unconditioned stimuli after a single injection of 5-HT; 5-HT (daily)—the snails which were receiving daily injections of 5-HT during 4 days; 5-HT (daily + L)—the snails, being trained after the daily injections of 5-HT; control—the naive snails; *Asterisk*—the reliable difference ($p < 0.001$) versus the control group

3.3 Experiment 3

A single injection of 5-HT also causes a depolarization shift in Vm of 5 mV ($n = 5$). After the initial stage of learning (10–12 pairs during 1 day), in the snails, which were given a single injection of 5-HT, there was a depolarization of Vm at 4.5 mV ($n = 10$) (Fig. 1).

The increase of neuron excitability under the direct action of 5-HT was noted by a number of authors [1, 11, 12]. The results demonstrate that under the action of 5-HT, the functional state of neurons varies, therefore, the efficiency of their influence on the neural network in which they belong changes. This means that the appearance of extracellular 5-HT, which can be released, for example, from the modulatory 5-HT-containing neurons [1, 5], can modulate the rate of learning, i.e., the result we have shown here. On the other hand, it is known that neuronal excitability is increased as a result of training, the fact known of the both cellular (membrane) correlates of learning [13–16]. It seems to us as an interesting result, showing that an increase in the excitability of neurons occurs already in the initial phase of training after releasing 5-HT. We plan to study in future experiments the dynamics of this process and its importance.

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