ISSN 1990-7478, Biochemistry (Moscow), Supplement Series A: Membrane and Cell Biology, 2023, Vol. 17, No. 4, pp. 264–268. © Pleiades Publishing, Ltd., 2023.
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Action of Serotonin Precursor Synthesis 5-Oxytryptophan on EPSP Recorded in Premotor Interneurons of Snail after Formation of Conditioned Defensive Reflex

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Received April 17, 2023; revised May 24, 2023; accepted May 29, 2023

Abstract—A quantitative study of subthreshold excitatory postsynaptic potentials (EPSP) recorded intracellularly in giant premotor interneurons of the terrestrial snail was carried out after the formation of a conditioned defensive reflex of food aversion in snails with increased level of serotonin. The results showed a significant increase in the number of low-amplitude EPSP with an amplitude from 0.3 to 0.5 mV in the giant premotor interneurons of defensive behavior after learning and increasing the level of serotonin. The observed increase in the number of EPSP may indicate either an increase in the number of action potentials in the corresponding presynaptic neurons or an increase in the amplitudes of the EPSP that were previously undetectable.

Keywords: neuron, training, EPSP, synaptic plasticity, membrane potential, serotonin

DOI: 10.1134/S1990747823050033

INTRODUCTION

Serotonin is one of the most widespread and most studied neurotransmitters of the nervous system [1, 2]. It plays an important role in the processes of learning and memory formation, and is involved in the modulation of the strength of the synaptic connection [3–6]. The aim of this research was to study the effect of the serotonin synthesis precursor 5-hydroxytryptophan (5-HTP), which leads to an increase in the level of serotonin in the body, on the subthreshold electrical activity of the giant premotor interneurons after formation of associative learning in terrestrial snail. Registration of subthreshold activity of a neuron allows us to describe the total electrical activity of incoming synapses. To achieve this goal, the following task was set: to analyze the changes in the subthreshold activity of premotor interneurons after the formation of a conditioned reflex of aversion to a certain type of food in a snail injected by 5-HTP.

MATERIALS AND METHODS

Formation of a conditioned defensive reflex of aversion to food (CR). For this experiment, healthy and active terrestrial mollusks *Helix pomatia* were selected. Before the start of the experiment, animals were kept in an active state for 14 days. Humidity and temperature necessary were controlled in the terrarium using measuring instruments. Carrots were used as food. During 3 days before the start and throughout the entire duration of the experiment, the animals were not receiving the food, that was a standard procedure in the formation of a defensive reflex of aversion to a certain type of food which is related to the need to maintain mollusks in an active state [4]. Then the animals were divided into four groups: experimental group 5-HTP + CR, experimental group 5-HTP, experimental group CR, and control group (intact animals). Animals of the first and second experimental groups were injected with the precursor of serotonin synthesis 5-HTP (n = 5) at a concentration of 1 mmol/L for 4 days. The pharmacological effect of 5pHTP in the body is a significant increase in the amount of serotonin already 30 min after injection, which reaches a maximum value after 1 h and lasts several hours [7].

The animals of the first experimental group received a 1-h training session after the injection of 5-HTP. Animals of the third group received the training session according to the same scheme as the animal of the first group without preliminary injection of 5-HTP. The CR was generated using the following technique: a piece of cucumber was presented on a metal rod to the oral region of the mollusk as a conditioned stimulus. At the moment of the first chewing movement of the snail, a current of 1–2 mA was passed through the rod as an unconditional stimulus causing a defensive reaction. Between combinations of food and shock,

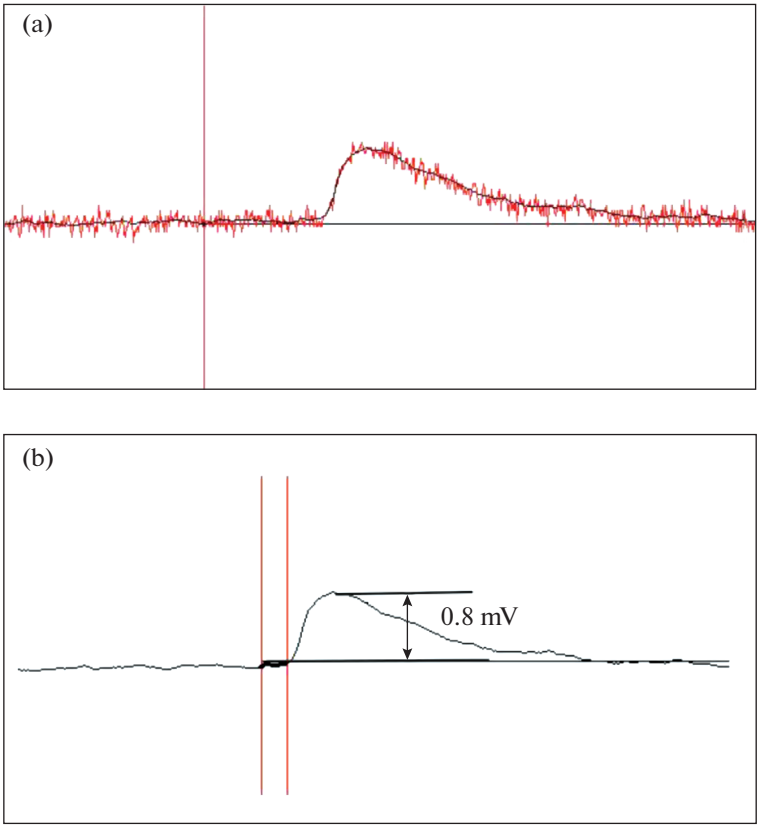

Fig. 1. An example of recording an EPSP (a) and a method for calculating its amplitude (b). Averaging over 100 points with a signal digitization step of 0.1 ms.

5–7-min pauses were made. Two training sessions with 10 combinations of conditional and unconditional stimuli were presented each day of formation of CR. To confirm the formation of the reflex, the animal was given a cucumber for 3 min, and if the snail refused to take a piece of cucumber during this period, then this reaction was taken as a refusal of this type of food. On average, the reflex was developed after the presentation of 80 combinations of stimuli over 4 days. Animals in the control group were kept intact under the same conditions as the experimental groups.

The studies were carried out on 22 snails: 5 snails were in a group of trained snails with 5-HTP application (the number of neurons with recorded activity, $n = 9$); 5 snails were in a group with only 5-HTP application ($n = 9$ neurons with recorded activity); 5 snails were in a group of snails in which a conditioned defensive reflex was formed ($n = 7$ neurons with recorded activity); 5 snails were in a group of intact animals ($n = 7$ neurons with recorded activity).

Electrophysiological experiments. Electrophysiological measurements were carried out according to standard methods at room temperature using one or two intracellular glass microelectrodes filled with 2.5 M KCl solution; diameter of a micropipette tip was about 0.5 μm, and microelectrode input resistance was 5–25 MOhm [8–11]. Microelectrodes were prepared from glass capillaries with an outer diameter of 1.5 mm on a special microforge. The microelectrode solution was connected to the recording equipment via a current-conducting "agar bridge–silver chloride electrode" chain. The indifferent electrode was a symmetrical chain, the agar end of which was placed into the solution washing the preparation. The microelectrodes were brought to the cells under visual control using a binocular microscope. The recording was made using a computer with a built-in analog-to-digital converter.

Parameters of the subthreshold activity of interneurons of the parietal ganglion of the snail. Electrophysiological measurements were carried out using the method of recording the transmembrane potential, which allowed the detection of excitatory postsynaptic potentials (EPSPs) with an amplitude of 0.3 mV. During the experiment, we analyzed synaptic input to premotor giant interneurons: LPa2, Lpa3, Rpa2, and Rpa3. EPSPs were determined visually by the characteristic of the change in membrane potential [11]. The parameters facilitating the registration of EPSPs were associated with achieving minimal noise, averaging the obtained registrations with the necessary averaging parameters, and selecting optimal display scales

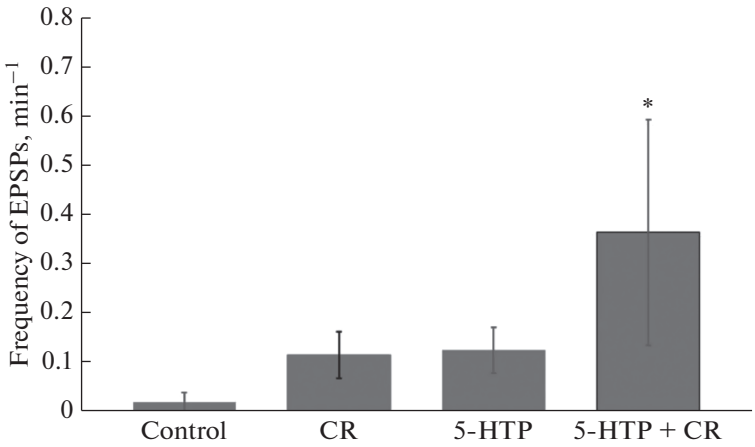

Fig. 2. The average value of the number of single EPSPs with an amplitude from 0.3 to 0.5 mV. *, The difference between the group and the control is statistically significant at $p < 0.05$.

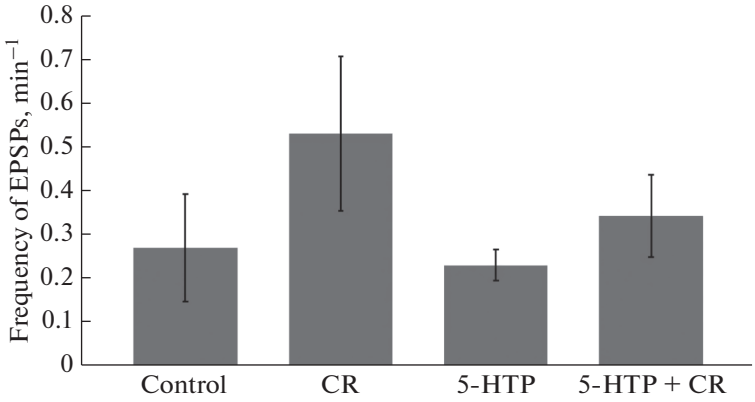

Fig. 3. The average value of the number of single EPSPs with an amplitude from 0.5 to 1 mV.

curves. Based on a series of long-term recordings of transmembrane potentials of silent premotor neurons, we have developed a technique allowing us to quantify the total synaptic influx of an individual neuron based on the membrane potential recordings lasting 30 min. To describe the observed changes in the membrane potential of interneurons, the following parameters of the synaptic background activity of parietal ganglion interneurons were chosen: average amplitude and average number of EPSPs. A value of 0.3 mV was taken as the threshold EPSP amplitude, as only this value allowed us to identify EPSPs at the noise level (Fig. 1).

Statistical analysis. Mean values of the measured parameters and standard errors of the mean are presented. The significance of differences between the means values of the control and experimental groups was checked using the nonparametric Mann–Whitney test. The Sigma Plot 11 software was used. Statistical significance was assessed at $p < 0.05$.

RESULTS AND DISCUSSION

Single EPSPs of various amplitudes were observed during the registration of subthreshold electrical activity of premotor interneurons. The frequency of EPSP appearance in the studied neurons were analyzed in groups of intact animals (number of neurons with recorded activity, $n = 7$), in trained animals ($n = 7$ neurons), animals after injection of 5-HTP ($n = 9$ neurons) and in animals that were trained after injection of 5-HTP ($n = 9$ neurons). All observed EPSPs were divided according to their amplitude into intervals: (1) from 0.3 to 0.5 mV, (2) from 0.5 to 1.0 mV, (3) from 1.0 to 1.5 mV, (4) from 1.5 to 2.0 mV, (5) from 2.0 mV to the threshold value. It was found that the frequency of occurrence of EPSPs with an amplitude from 0.3 to 0.5 mV was significantly ($p < 0.05$) increased in the group of animals that were trained after injection of 5-HTP (Fig. 2). There was also an unreliable tendency for an increase in the number of EPSPs in the group of trained snails with injection of 5-HTP compared with the group of animals which had only injection 5-HTP, and which was only trained. No significant changes in the frequency of occurrence of EPSPs of other amplitudes were found (Figs. 3–5). Thus, our experiments showed that injection of 5-HTP before training led to a significant increase in the frequency of occurrence of EPSPs with an amplitude from 0.3 to 0.5 mV. At the

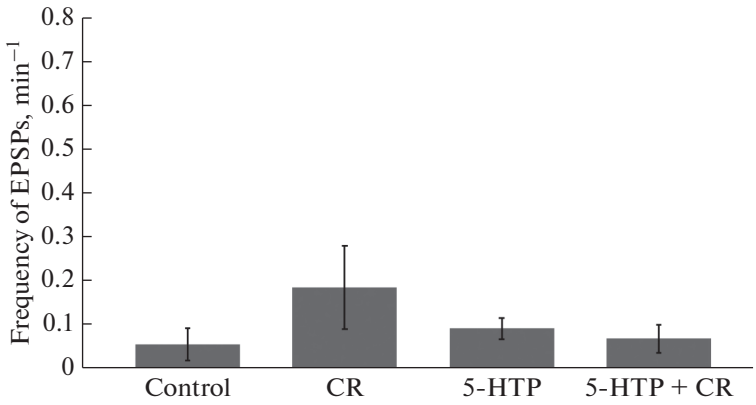

Fig. 4. The average value of the number of single EPSPs with an amplitude from 1 to 1.5 mV.

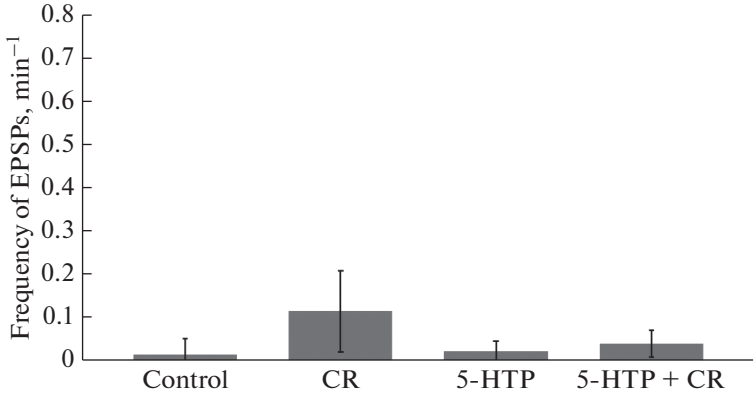

Fig. 5. The average value of the number of single EPSPs with an amplitude from 1.5 to 2 mV.

same time, an unreliable tendency towards an increase in the number of EPSPs of this amplitude interval was observed when training accompanied with injection of 5-HTP compared with development of a conditioned defensive reflex of aversion to food, as well as with separate injections of 5-HTP. Probably, this increase in the number of low-amplitude single EPSPs indicated either an increase in the number of action potentials in the corresponding presynaptic neurons, or an increase in the amplitude of EPSPs that were previously undetectable. Our analysis of the subthreshold background activity of silent premotor interneurons of the defensive behavior of the snail allowed us to find changes in the synaptic input associated with the influence of the precursor of serotonin synthesis 5-HTP, as well as with the development of a conditioned defensive reflex of food aversion against the background of 5-HTP application.

FUNDING

This work was supported by the Strategic Academic Leadership Program of Kazan Federal University (PRIORITY-2030).

COMPLIANCE WITH ETHICAL PRINCIPLES

The authors declare that they have no conflict of interest.

All experimental procedures were performed in compliance with the rules and regulations of the ethical committee of the Kazan Federal University.

REFERENCES

1. Losonczy A., Makara J.K., Magee J.C. 2008. Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature*. **27** (452), 436–441.
2. Lisman J., Cooper K., Sehgal M., Silva A.J. 2018. Memory formation depends on both synapse-specific modifications of synaptic strength and cell-specific increases in excitability. *Nat. Neurosci*. **21** (3), 309–314.
3. Bliss T.V., Lomo T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol*. **2** (232), 331–356.
4. Balaban P.M., Korshunova T.A. Network, cellular and molecular mechanisms of plasticity in simple nervous systems. Successes of physiol. *Science*. **42** (4), 3–19.
5. Gainutdinov H.L., Andrianov V.V., Gainutdinova T.H. 2011. Changing the excitability of the neuronal membrane as a cellular mechanism of learning and memory. *Uspekhi fiziologicheskikh nauk* (Rus.). **42** (1), 33–50.

6. Andrianov V.V. Bogodvid T.K., Deryabina I.B. 2015. Modulation of defensive reflex conditioning in snails by serotonin. *Front. Behav. Neurosci*. **9**, 279.
7. Naumenko E.V., Popova E.K. 1975. *Serotonin and melatonin in the regulation of the endocrine system*. Novosibirsk: Nauka.
8. Gainutdinov H.L., Andrianov V.V., Gainutdinova T.H., Tarasova E.A. 1998. Electrical characteristics of command and motor neurons in the development of a conditional defensive reflex and the formation of long-term sensitization in snails. *Zhurnal vysshei nervnoi deyatelnosti* (Rus.). **48** (6), 1004–1013.
9. Deryabina I.B., Muranova L.N., Andrianov V.V., Gainutdinov K.L. 2018. Impairing serotonin synthesis by *p*-chlorphenylanine prevents the forgetting of contextual memory after reminder and the protein synthesis inhibition. *Front. Pharmacol*. **9**, 607.
10. Palikhova T.A., Marakueva I.V., Arakelov G.G. 1992. Mono- and polysynaptic connections between identified neurons in the passive-defensive reflex system of the grape snail. *Zhurnal vysshei nervnoi deyatelnosti* (Rus.). **42** (6), 1170–1179.
11. Balaban P.M., Zakharov I.S. 1992. *Training and development. The common basis of two phenomena*. Moscow: Nauka.

Translated by A. Arslanov

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