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Effects of Monodiets with Different Tryptophan Contents on Shell Color, Behavior, and Neuron Activity in the Common Snail

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The essential amino acid tryptophan is a precursor of serotonin, one of the most important neurotransmitters in the brains of vertebrates and the central nervous systems of invertebrates. Extensive data have been obtained on the effects of exogenous serotonin and its precursors on behavior and neuron activity. Less well studied is the influence of tryptophan in food products. This report presents results from studies of the effects of long-term monodiets containing different quantities of tryptophan on the visual and behavioral characteristics of the snails *Helix pomatia* and *Helix lucorum*, which are mollusks, and on the electrical activity of identified neurons in these species.

Keywords: tryptophan, serotonin, common snail, monodiet, behavior, electrical activity, identified neuron.

Introduction. This article presents results from studies of the influences of long-term monodiets on the visual and behavioral characteristics of the common snail and electrical activity in identified neurons in these neurons. The monodiet selected for study was based on the food content of the essential amino acid tryptophan. Tryptophan is a precursor of 5-hydroxytryptophan (5-HTP), which is the direct precursor of serotonin (5-hydroxytryptamine, 5-HT). Dietitians recommend diets and biologically active supplements (BAS) containing products with large quantities of tryptophan and 5-HTP to increase tone and improve status in depression, memory impairment, and sleep. Biochemical data on the contents of various amino acids in food products have been obtained [Skurikhin and Volgarev, 1987]. Historical reports on health impairments in tryptophan deficiency have been presented [https://lahtaclinic.ru/article/pellagra/]. However, not all doctors are convinced of the efficacy of tryptophan ingested with food [http://dietolog.org/components/tryptophan/] in correcting behavior, so use of tryptophan and 5-HTP as biologically active supplements (BAS) is advised. Use of diet and BAS is based on data on the mechanisms of action of serotonin and its precursors, including at the neuron level.

In neurobiology, serotonin is regarded as one of the most important neurotransmitters; it is involved in cognitive processes, including functional status, attention, emotions, and memory. However, simultaneous study of the influences of exogenous serotonin precursors at the behavioral and neuronal levels in higher animals is difficult. Model systems for these studies include invertebrates. Thus, the Nobel Prize in Physiology and Medicine in 2000 was awarded to Eric Kandel for advances in studies of learning processes at the neuronal level and serotonin as a necessary element of learning systems [Kandel, 2000]. Detailed reviews of the literature on the influences of exogenous serotonin and its precursors on behavior and neuron activity in invertebrates have been published in a compendium [D'yakonova and Sakharov, 2019] and various articles [Sakharov, 1990; Tsyganov and Sakharov, 2002]. Sakharov used experimental data as the basis of the concept of the "heterone" [Sakharov, 1990]. Data on the role of serotonin in learning were obtained in *Aplysia* and the common snail [Kandel, 1980; Balaban, 2017; Kandel, 2009; Kandel, 2000; Balaban, 2002]. Most reports describe studies of the functions of serotonin and its precursors given exogenously. The aim of the present work was to compare behavioral indicators and

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intracellular recording results in conditions of natural ingestion of tryptophan with food in common snails kept on monodiets for prolonged periods.

Methods. It is difficult to obtain data on the neuronal mechanisms of action of dietary tryptophan in higher vertebrates, so we used the terrestrial pulmonate mollusk – common snails – as a model system. Common snails – *Helix pomatia* and *Helix lucorum* – constitute a well studied object in neurobiological research [Balaban, 1987; Sakharov 1992]. The main advantage, among others, of using snails is that they have identified neurons, such that the electrical activity of individual neurons can be compared in different experiments [Ierusalimskii et al., 1992].

The basis for selecting a monodiet for keeping snails for prolonged periods (up to two years) from products common to humans and snails was tryptophan content [Skurikhin and Volgarev, 1987]. Thus, three widely distributed plant products were used: carrots, bananas, and apples. Data from a table in the Nesterin and Skurikhin [1979] compendium show that bananas and carrots contain 45 and 42 mg tryptophan per 100 g of product, compared with 12 mg/100 g for apples. The tryptophan contents in carrots and applies differ by a factor of 3–4. Bananas for the third experimental group of animals were selected to confirm the possible influence of factors other than tryptophan on the study parameters. In addition, there is significant divergence in the literature for bananas. Snails received no other foodstuffs during the study.

Behavioral study. Studies in different invertebrates haven demonstrated a relationship between activity and the endogenous serotonin level [Balaban, 1987; Sakharov, 1990]. In our experiments, levels of activity (five levels – from being inside the shell to being maximally active with the tentacles extended) were compared by systematic observation of the three groups with results being recorded in data tables. The snails' activity was also assessed in terms of the threshold of arousal of the snails in response to electrical stimulation via paired silver electrodes attached to the shell. Groups of snails kept on monodiets were designated C (carrots), B (bananas), and A (apples). In addition, the positions of "sleeping" snails in aquariums were noted (level 0) in relation to the light source (above–below). Statistical analysis of the data (computation of mean values, deviations from the mean, errors of the mean, and significant differences using Student's test) was run in Microsoft Excel 97-2003.

Neuron activity. After behavioral verification, snails were prepared for electrophysiological studies. Two types of semi-intact preparations were used: "split leg" and "CNS-viscera." Neuron electrical activity was recorded using intracellular glass microelectrodes filled with potassium chloride or citrate. Electrical stimulation of the mantle ridge was via paired electrodes with pulses (50 msec) from a stimulator. Intracellular stimulation was applied through the recording microelectrode using a bridge amplifier scheme (MES-8201, Nihon Kohden). Recorded signals, after amplification and digitization, were recorded in the Axon program and analyzed in Mini Analysis Program (Synaptosoft 6.0.7., by Justin Lee, 1997–1999).

Results. *Visual parameters*. Shell color and pattern intensity were significantly different in snails kept for prolonged periods on the carrot (C), banana (B), and apple (A) monodiets. A blinded test for visual differences used random observers (about 20) and asked them to divide the snails into groups (two groups each of seven snails), which they did error-free for groups consisting of dormant snails kept on the C and B diets. The shells of banana snails were significantly lighter, while species differences in the coloring of *H. pomatia* (Moscow area) and *H. lucorum* (Crimea) were almost completely absent [Palikhova, 2016]. A further characteristic of snail of the B group was the presence of water-insoluble colored material in their droppings. It can be suggested that this is linked with differences in carotene-utilizing enzymes, though biochemical analysis was beyond the remit of this study. Shell color differences for C and A snails were not observed.

Behavior, activity. No unambiguous relationship between keeping snails on the C, B, and A diets and their activity was seen. In terms of position in the terrarium, there was a minor preference of snails of the B group to be in the more illuminated upper part. No relationship was seen between the level of activity in snails kept on the carrot and banana monodiets (T test 0.6, $p \leq 0.05$). No differences in the arousal threshold were seen (8.1 and 7.6 V, $n = 20$).

Intracellular neuron activity. Neuron activity was recorded from the parietal ganglia of 56 snails kept for more than six months on the C (28), B (17), and A (27) diets. Neuron activity was compared using standard parameters: membrane potential (MP, mV), presence of baseline spike activity and its type (synaptic and/or pacemaker action potentials (AP), A-spikes), and thresholds of action potential generation and pacemaker spike activity (nA). The occurrence of responses to mechanical and/or electrical sensory stimuli was verified. The following were determined: MP (mV), AP threshold (nA), pacemaker spike activity threshold (nA), and maximum amplitude of total synaptic response to electrocutaneous stimulation (tEPSP, mV) (Fig. 1).

No significant differences were seen between neuron activity in C, B, and A snails. Thus, the mean MP of neurons in all groups was about 50 mV (Fig. 1, *a*). Neurons known as defensive behavior command neurons [Balaban, 1987] were typically silent, with AP thresholds of 1–4 nA, greater intracellular currents generating spike pacemaker activity. The spread of mean threshold values for neurons in the three groups of snails could be explained in terms of differences between identified neurons in the subglottal complex of ganglia, not going beyond the limit of deviations from the mean (Fig. 1, *b*). Synaptic potentials arose both in baseline conditions and in response to mechanical and electrical sensory stimulation of different parts of the body in semi-intact snail preparations (total excitatory postsynaptic potentials (tEPSP)). The parameters of evoked responses

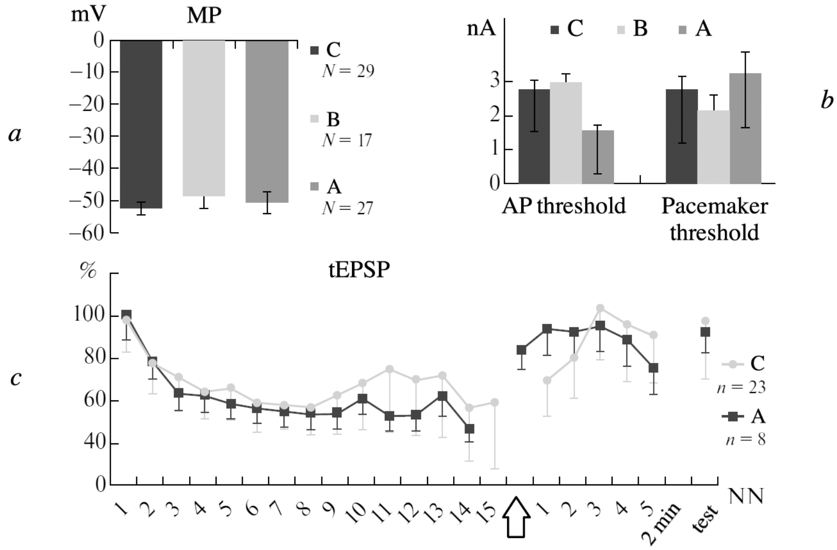

Fig. 1. Electrical activity of neurons in three groups of snails (C – carrot diet, *n* = 29; B – banana diet, *n* = 12; A – apple diet, *n* = 27). Neurons of the three groups were compared in terms of membrane potential (*a*) and intracellular current (nA) thresholds for AP generation and pacemaker spike activity (*b*). *c*) Overall plots showing acclimation of tEPSP amplitude in series of rhythmic (0.1 Hz) electrical sensory stimulation of the mantle ridge. The heterosynaptic potentiation effect was recorded in 23 neurons in the C group and eight in the A group. The external stimulus (arrow) consisted of injection into the foot in a series of rhythmic stimuli to the mantle; data spreads are shown as errors of the mean. Recovery of neuron responses were tested (test) at 2 min (2 min) after series of rhythmic stimuli.

were no different from known parameters [Ierusalimskii et al., 1992]. Large neurons in the ventral area of the parietal ganglia showed baseline activity typical of these neurons, of the pacemaker, synaptic, and mixed types. Neurons RPa6, RPa7, and LPa6 showed axon spikes typical of these cells (A-spikes).

As the influences of serotonin are believed to be linked to its involvement in learning processes [Balaban, 2017; Kandel, 2009], we tested well-known phenomena of the plasticity of synaptic responses of parietal neurons [Palikhova et al., 2019; Sokolov and Palikhova, 1999]. Preliminary data demonstrated typical dynamics of acclimation of overall synaptic responses. Less unambiguous results were obtained on the influences of suprathreshold heterosynaptic stimulation on tEPSP amplitude in series of rhythmic electrical stimulation of the mantle ridge. The heterosynaptic potentiation effect [Abramova et al., 2007; Palikhova and Pivovarov, 2016] was seen in 23 of 29 neurons in C snails and eight of 27 neurons in A snails. Heterosynaptic potentiation occurred more frequently in neurons of the C group (about 80%) than the A group (about 30%). However, these are preliminary data and require verification, as the injection into the foot used as the external stimulus is difficult to standardize. We can say confidently that heterosynaptic potentiation was recorded in neurons of both groups of snails, albeit with different frequencies and dynamics (Fig. 1, *c*).

Discussion and Conclusions. Thus, our studies found no significant differences either in behavioral criteria or neuron activity in snails kept on different monodiets for prolonged periods. Apples contain 3–4 times less tryptophan than carrots and bananas. Nonetheless, clear external differences (shell color) were seen only in snails on the banana monodiet [Palikhova, 2016]. This may be linked with the detail of carotene metabolism, though biochemical analysis was outside the scope of the present study. Results obtained from intracellular investigations of identified snail neurons were no different for snails from the three monodiet groups or from previously reported data [Ierusalimskii et al., 1992]. Our data confirm the views of some doctors regarding the lack of importance of dietary tryptophan for supporting normal serotonin metabolism [https://healthylife.nsp.com/], though we do not share the conclusion made by these authors, that consumption of BAS is required. There is potential in continuing studies of neuronal plasticity to explain the data on the role of exogenous tryptophan in individual differences in short-term memory and attention processes.

Thus, we found no significant differences between snails kept for prolonged periods on monodiets containing different quantities of tryptophan, either in behavioral criteria or in terms of measures of the activity of identified neurons.

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