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ABSTRACT

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Abstracts

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unpredictable change in defensive and motor activity. It was determined that during the formation of long-term sensitization, which is close reaction such as fear, there is an increase in the excitability of the main elements of the neural network: sensory and command neurons, as well as an increase in the amplitude of EPSP. (Hochner et al., 1986). At the same time, it seems interesting how change the characteristics of neurons involved in the performing of defensive and motor responses in mollusks during anxiety. The aim of present study was to investigate how the anxiety-like behaviour affected the membrane characteristics of premotor interneurons of defensive reflex in snails.

The experiments were carried out on the terrestrial mollusk Helix Pomatia. To develop an anxiety-like behavior, 4 electrical stimuli were applied to mollusk on the area of the head each day with an interval of 1.5 hours within 3 days. Then the animals had 3 days of rest and after the same series of electrical stimulations were repeated. Anxiety-like behaviour was determined by behavioral tests such as locomotion, ommatophore and pneumostom retraction time. The electrophysiological activity of premotor interneurons was recorded on an isolated preparation of the nervous system of snails. The animals of the control group were kept in identical conditions, as well as the animals of the group when an anxiety-like behaviour was formed. The following parameters of the membrane characteristics of premotor interneurons were studied: membrane potential (Vm), amplitude and duration of excitatory postsynaptic potentials (EPSP). The registration of electrophysiological characteristics in snails that did not undergo the formation of anxiety-like behaviour was a control.

The study of the membrane characteristics of premotor interneurons showed that the membrane potential of premotor interneurons of snails with anxiety-like behaviour significantly shifted towards depolarization: Vm of the interneurons of animals with anxiety-like behaviour was -52.95±1.7 mV (n=9), while the Vm of the interneurons of animals in the control group was 59.2±2 mV (n=6) (P<0,05).

An analysis of the EPSP activity of premotor interneurons in snails showed that the total amplitude of EPSP recorded from these neurons in anxiety snails (1,01±0,1 mV) was slightly reduced compared to the total amplitude of EPSP in the premotor neurons of animals in the control group (1,3±0,3 мВ). The frequency of EPSP appearance in premotor interneurons in the group of animals with anxiety-like behaviour did not differ from that in the interneurons of control animals.

Thus, obtained data showed that, the rest membrane potential of premotor interneurons of defensive reflex in snails with anxiety-like behaviour was more depolarized compare with rest membrane potential in premotor interneurons in control snails, while the parameters of EPSP which reflected the activation of synapses from sensory neurons do not change significantly. The tendency to decreasing EPSP amplitude after the formation of an anxiety-like behavior can be explained by the depolarization of the membrane potential against which a part of the EPSP signal is lost. The mechanisms of the depolarization shift of the resting membrane potential during the formation of an anxiety-like state requires further study and is the goal of our next work.

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S9.656. Membrane correlates of learning in molluscs: the role of serotonin, glutamate, and nitric oxide in the formation of conditioned defensive reflexes in the grape snail

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Processes of learning and memory underlie behavior change, and memory is one of the main cognitive functions of the brain. The mechanism for storing and/or remembering the received information constitutes memory. The issues of memory consolidation, including the formation of conditioned reflexes, remain relevant. Although the question about the mechanisms of learning and memory arose a long time ago, it has not yet been fully studied. Neuromodulation can have a significant impact on the formation of long-term memory [1]. Examples of neuromodulators in the simple nervous system of mollusks are serotonin, nitric oxide, and glutamate. The literature demonstrate that serotonin (5-HT) is the main mediator that modulate defensive behavior in mollusks. 5-HT, applied to the surrounding solution, causes several cellular changes that lead to an increase in the defensive reflex. In addition to the well-known role of 5-HT as a mediator in synaptic transmission, it was shown that it can perform integrative functions when released into the extracellular environment [1]. These results served as the basis for the application of 5-HT washing solution as a reinforcing stimulus for the purpose of creating cellular analogs of learning. By applying 5-HT to the solution washing the central nervous system, it is also possible to reproduce the electrophysiological correlates of plasticity.

Nitric oxide (NO) is known as one of the most important signaling molecules regulating the physiological functions of the body and cell metabolism. Much attention is drawn to the study of the role of NO in the mechanisms of learning and memory. NO-synthesizing neurons have been found in the nervous system of invertebrates, including mollusks. In mollusks, as in mammals, NO plays the role of an intercellular messenger and a signaling molecule in various parts of the nervous system. We have shown that both the NO donor sodium nitroprusside and the NO-synthase blocker L-NAME have a direct effect on the electrical characteristics of the premotor interneurons of the terrestrial snail. It is known that an essential role in the regulation of brain activity, particularly in memory processes, is played by L-glutamate, the main excitatory neurotransmitter in both vertebrates and many invertebrates. On the one hand, we studied the effect of changes in the content of serotonin, nitric oxide and glutamate on the formation of conditioned defensive reflexes of aversion to food and changing the environment, as well as on the reconsolidation of memory of these reflexes. On the other hand, we have conducted studies of the membrane mechanisms of the formation of conditional defensive reflexes in a mollusk with a simple nervous system – the terrestrial snail. To do this, we analyzed changes in the excitability of the premotor interneurons of the defensive reflex LPa3 and RPa3: the values of the membrane potential (Vm) and the threshold of action potential generation (Vt).

It was found that the application of 5-HT and the precursor of its synthesis 5-hydroxytryptophan (5-HTP) into the washing solution caused a decrease in the membrane potential (Vm) of LPa3 and RPa3 neurons, in both intact and trained animals. At the same time, in trained and sensitized snails, unlike intact snails, this application caused an increase in threshold potential (Vt). The results show that the responses (sensitivity) of premotor interneurons to extracellularly applied 5-HT or 5-HTP change after associative learning and long-term sensitization. It has been demonstrated that the reconsolidation of this contextually dependent memory of the situational conditioned reflex (CR) during reminder and simultaneous inhibition of protein synthesis does not occur if serotonin transmission is disrupted in the nervous system. It is shown that the development of the CR to the situation is accompanied by a depolarization shift and a decrease in the Vt of LPa3 and RPa3 neurons. No further Vm changes were detected after the reminder (initiation of reconsolidation) both with the subsequent injection of a protein synthesis blocker or saline solution. The Vt of these neurons decreases after learning and remains unchanged after the initiation of reconsolidation.

It was found that blocking the NMDA receptor with the MK-801 blocker in terrestrial snails accelerates the process of aversive learning. It has been shown that the application of a NO sodium nitroprusside donor into a solution washing the preparation of intact snails causes an increasing hyperpolarization of the membrane of premotor interneurons at 5.5 mV by the 10th minute. The application of the L-NAME

NO-synthase blocker into the solution washing the isolated snail preparation caused a gradual decrease in the membrane potential by 5.0 mV for 30 minutes. Thus, we have demonstrated that in certain neurons NO synthesis inhibition (i.e. a decrease in its amount) can cause depolarization of the membrane whileincreasing NO causes hyperpolarization. This allows us to assume the correlation of the NO level in a neuron with its membrane potential. The obtained results also indicate the need for 5-HT for the process of reconsolidation of memory in the example of a grape snail.

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S9.657. Metabolic imaging of liver tissue in the process of regeneration with pathology

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More than 1 million new cases of primary and secondary liver cancer are registered annually. The only type of therapy remains surgical treatment of the liver. However, 5-year survival after liver resection reaches 14-61%. Despite modern advances in surgical technique and the improvement of methods for preoperative assessment of liver function, there is still a high risk of postoperative liver failure, associated with the presence of background hepatic pathology, as well as with the difference in the regenerative potential of the liver of each patient. Standard clinical methods for assessing the state of the liver tissue are not sufficiently informative and do not allow a predictive assessment of the regenerative potential of the liver remnant. Thus, the search for new methods and criteria for intraoperative rapid assessment of the structure and function of the liver remains an urgent task, which would make it possible to detect the presence of pathological changes already in the early stages, as well as to assess the regenerative potential of the liver remnant. Modern label-free methods of multiphoton microscopy with time-resolved microscopy (FLIM) and optical second harmonic generation (SHG) expand the possibilities of studying the structural and functional state of liver tissue at the cellular level.

A series of experiments were carried out on Wistar rats. Normal regeneration was induced by 30% and 70% partial hepatectomy (PH). Fibrosis was induced by administration of CCl4 for 8 weeks. Steatosis was induced with a 60% high fat diet for 12 weeks. At different stages of the pathology, we induced regeneration by 70% PH. Monitoring of the regenerating liver was carried out on days 3rd and 7th after PH. Using multiphoton microscopy, we performed analysis of the liver tissue structure. The assessment of collagen content in the tissue was carried out by the SHG. Using FLIM, we analyzed the contributions of the fluorescence lifetimes of bound forms of NADH (a2,%) and NADPH (a3,%). Morphological and morphometric analysis and a standard biochemical blood test were performed as control methods.

As a result, we revealed that the regeneration of normal liver is characterized by a uniform distribution of the NAD(P)H autofluorescence signal and the absence of collagen accumulation in the tissue. The FLIM method revealed the main optical criterion for successful regeneration - a sharp increase in the contributions of a2 and a3 on the 3rd day of regeneration, which is associated with an increase in the intensity of oxidative phosphorylation and biosynthetic processes in hepatocytes. In case of induced steatosis and fibrosis, we identified zones with a reduced NAD(P)H autofluorescence signal associated with foci of fibrosis and lipid infiltration. Using FLIM we showed a decrease in the contributions of a2 and a3 in the early stages of pathology, with a subsequent increase in these parameters in the later stages. With the induction of regeneration at the later stages of the pathology, there is no jump in a2 and a3 on the 3rd day of regeneration, which is associated with lipotoxicity and mitochondrial dysfunction in hepatocytes. Thus, we identified the optical criteria for successful liver regeneration: a uniform distribution of the NAD(P)H autofluorescence signal, as well as a sharp increase in the contributions of the fluorescence lifetimes of the bound form of NADH and NADPH. The criteria for violated liver regenerative potential in the presence of pathology defined by zones with a reduced NAD(P)H autofluorescence signal, as well as the absence of a sharp jump in the contributions of the bound form of NADH and NADPH at the later stages of pathology. The results obtained could be useful for the developing criteria for a predictive express-assessment of the state of the liver tissue in the clinic.

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S9.658. Mitochondrial pore (mPTP) and cell death

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Various pathological conditions such as neurodegeneration, cardiovascular diseases, cancer and aging are associated with impaired mitochondrial function and, in particular, mitochondrial pore (mPTP). The opening of mPTP leads to a acute increase in the permeability of the mitochondrial membrane, loss of mitochondrial potential and triggering programmed cell death – apoptosis or necrosis. In turn, the opening of mPTP may be caused by ROS overproduction, calcium overload, low substrate availability, inhibition of mitochondrial respiratory complexes, etc. It is assumed that the mechanism of triggering apoptosis mediated by mPTP is disrupted in tumor cells, while in neurodegeneration, the toxicity of protein aggregates, such as a-synuclein, is mediated by the opening of mPTP.

It is known that the opening of mPTP is a universal mechanism of induction of apoptosis for various types of mammalian cells, however, the quantitative analisis of mitochondria with opened mPTP vs. total pool of mitochondria of the cell necessary to trigger cell death has not yet been established.

To study this parameter, we selected 4 mammalian cell types: neurons, astrocytes, breast cancer cells, and fibroblasts. The cells were loaded with a potential-sensitive TMRM dye (25 nM) and a fluorescent substrate of caspase 3 NucView (2 uM). Next, a step-by-step application of ferutinin (an electrogenic ionophore inducer of mPTP opening), mediating an increase in the permeability of the mitochondrial membrane and calcium overload of mitochondria, was carried out. The opening of mPTP occurs if the addition of ferutinin is accompanied by a rapid loss of mitochondrial potential and the extinction of TMRM fluorescence. The percentage of mitochondria with opened mPTP during the induction of apoptosis (which was evidenced by a sharp increase in NucView