The data of the behavioral experiments attest to the fact that rats are capable of differentiating the probabilistic characteristics of environmental signals (in particular, of reinforcement). Moreover, destruction of the frontal divisions of the rat cerebral cortex leads to a deficit in this type of behavior [4].

Thus, the reflection of the processes of probabilistic prognostication in the activity of neurons of the medial frontal cortex of rats points to the participation of this region in the prognostic activity of the brain.

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FEATURES OF ASSOCIATIVE LEARNING OF SMALL ISOLATED NEURONS OF THE EDIBLE SNAIL

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Neurophysiological experiments have demonstrated that at the beginning of training not all neurons are indifferent to the effect of the conditional stimulus, since the animal perceives the actuating signal in relation to which the conditioning will be carried out [3]. The facts establish that sensory cells should be such neurons [3, 5]. What does the associative learning effect consist of in such neurons? A modification of the initial response to the conditional stimulus has been found in the form of the widening of the action potential (AP), which creates favorable conditions for the formation of a spike in the subjacent motor neurons [4, 5]. The increase in the duration of the AP of the sensory neuron hypothetically leads to an increase in the amount of mediator secreted from the presynaptic terminal of the sensory neuron and reaching the motor neuron. At the present time not only has the phenomenon of the widening of the AP of the sensory neuron been investigated in detail, but the biochemical mechanisms which could support this exceptionally important biological process, as well [5]. Nevertheless, the origin of this phenomenon is unclear, that is, whether it is associated with a presynaptic or an endoneuronal mechanism, since in all studies previously carried out, even if one of the stimuli (usually the conditional) was targeted directly on the sensory neuron, while the second arrived through the synaptic contact, this left open the possibility for the formation of a presynaptic pathway, providing for the increase in the duration of the AP.

Results which were obtained in the neurons of the edible snail in conditions completely excluding the influence of presynaptic structures (the neurons were isolated) are presented in this report [1]. The possibility was investigated of the associative learning of small (less than 30 μm) neurons in response to the action of two suprathreshold electrical stimuli eliciting stable AP.

The method of preparing completely isolated neurons was previously presented in detail [1]. Neurons extracted from the right superior quadrant of the left parietal ganglion, in which sensory cells are located [2] were selected for the experi-

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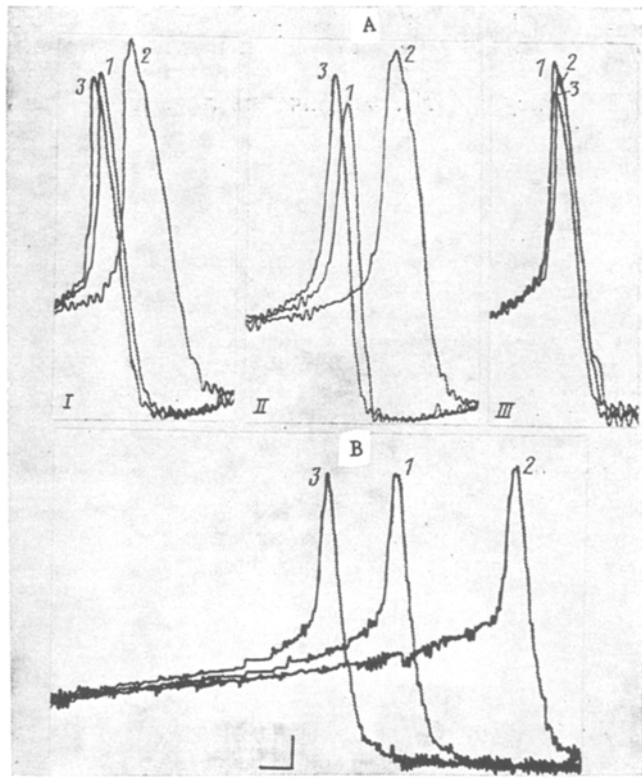

Fig. 1. Influence of repeated presentations of CS-US combinations on the duration of the AP and the latency period of their development. A) Influence of CS-US combinations on the duration of the AP. The CS is 0.18 nA, 300 msec; the US, 0.22 nA, 200 msec; the interval between the CS and the US, 50 msec; the frequency of presentation, once every 40–70 sec. The changes in the duration of the AP following the first (I) and third (II) series are shown. 1) An AP prior to the beginning of the series of CS-US presentations; 2) an AP after 10 CS-US presentations; 3) an AP 15 min after training. III) Influence of isolated presentations of the CS, not reinforced by the US, with an frequency of once every 40–70 sec, on the form of the AP. 1) An AP prior to the beginning of training; 2) an AP after 10 CS presentations; 3) an AP after 20 CS presentations. B) Change in the latent period of AP development after CS-US presentations. The CS is 0.8 nA, 300 msec; the US, 0.75 nA, 300 msec; the interval between the CS and the US, 50 msec; the frequency of presentation, once in 80 sec. 1) An AP prior to training; 2) an AP after 17 CS-US presentations; 3) an AP after a 15 min post-training break. Calibration: 10 msec; 10 mV.

ments. The results were obtained in 18 completely isolated small neurons. Electrical intracellular stimulations with depolarizing pulses through microelectrodes introduced into the cells served as the conditional (CS) and unconditional (US) stimuli. The duration of the electrical pulses was 50 to 100 msec, the strength of the current was from 0.1 to 2 nA, the interval between the CS and the US was 50 msec, and the frequency of the presentation of the CS-US pairs was once in 1–2 min. Three to six series were carried out in each neurons, containing 5–15 CS-US pairs each. A fourth microelectrode was introduced into the neuron for the presentation of test stimuli in control experiments in order to establish the specificity of the conditional stimulus.

Following their analysis on a D3-28 microcomputer, the results of the experiments showed that an increase in the duration of the AP or a decrease in the rate of its development, as well as an increase in the duration of the AP with a simultaneous decrease in the rate of its development, are observed following the associated CS-US presentations. The widening of the AP which appears following the presentation of CS-US combinations was found in 14 out of 18 neurons, and in eight cases it was accompanied by a decrease in the rate of its development of the AP (Fig. 1A). The widening of the AP increases following the performance of each successive series (Fig. 1A). The maximal widening of the AP is apparently determined by the conditions under which the experiment is carried out, and by the characteristics of the neuron under investigation. The increase in the duration of the AP is found immediately following the carrying out of the CS-US presentations, and is maintained for 5–15 min. The increase in the duration of the AP is 5–15 msec, with an initial width of 10–17 msec in normal physiological solution, when measured from the generation threshold to the rising front of the AP to the corresponding point on the descending front. The standard error of the mean in 22 series five minutes after training was 161 ± 26% ($p < 0.05$) for the associated CS-US, and 98 ± 5% ($p < 0.05$) for the nonassociated stimuli, when the CS was presented with the frequency of presentation of the CS-US combination. A break in the application of the CS-US leads to a gradual decrease in the duration of the AP (Fig. 1A). A microelectrode was introduced into the neuron in five experiments, for the application of test stimuli to check the specificity of the conditioned response in relation to the combination presented, and for the investigation of the dynamics of the transformation of the response in them before and after the training of the neuron. Analysis of the results of the control experiments showed that no significant changes take place in the duration of the AP. Similar results were also obtained when the form of the AP evoked by the US was studied. Consequently, the widening of the AP is specific in relation to the actuating CS-US combination.

The presentation of the CS-US combination led to a significant decrease in the rate of development of the AP in four neurons, which was expressed in an increase in the latent period simultaneously with the slowing of the rate of their generation (Fig. 1B). The increase in the latent period reached 45–60%. At the same time, a change in the duration of the AP was not found. The altered latent period was maintained for 5–10 min following training. Following the application of the CS-US, six neurons demonstrated a change in the rate of generation of the AP, and its widening (Fig. 1A). However, in this case the decrease in the rate of development of the AP was not so significant as in the neurons of the second group, and was 10–20% of the initial.

It is natural that the study of the ionic mechanisms of the development of the phenomenon of the widening of the AP and the slowing of the rate of their development excites significant interest. The substitution of solutions with a deficiency of calcium ions or sodium for normal physiological solution has been utilized experimentally. Preliminary results have shown that the absence of calcium ions does not prevent the formation of the phenomenon of the widening of the AP after the training of the neuron, although the form of the AP in a calcium-free solution was significantly altered even before the presentation of CS-US combinations. The deficiency of sodium ions proves to be critical for the formation of the phenomenon of the slowing of the rate of the generation of the AP, which has originated [sic] as the result of training, while the widening of the AP following the CS-US presentation was disturbed only by the addition of serotonin to the physiological solution in a concentration of 10^{-5} M [1]. In this case, the initial AP widened after 2–3 min to the degree that training no longer influenced the form of the AP.

The experiments conducted demonstrate that repeated application of two superthreshold associated stimuli leads in small neurons to an increase in the duration of the AP and a slowing of the rate of its development. Since the neurons were isolated, it is evident that the nature of this phenomenon can only be endoneuronal, and its 100% replication in certain neurons excludes the possibility of an artefact. The comparatively brief persistence in time (in the isolated CNS the phenomenon appears over the course of several hours [3]) is a feature of the widening of the AP obtained, as compared with the similar event described in studies on *Aplysia* [3-5]. Nevertheless, the formation of widened AP in the isolated neuron establishes the fact of the endoneuronal origin of this phenomenon in the process of conditioning.

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