MOLECULAR—CELLULAR MECHANISMS OF LEARNING OF THE COMMON SNAIL

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The development of sensitization and an associative habit of rejection of a specific type of food is accompanied by short-term and long-term changes in behavior, bioelectrical activity, and the dynamics of the content of Ca$^{2+}$ in the command neurons of defense behavior of the common snail. In approximately an hour from the moment of the beginning of training, behavioral and neurophysiological effects which were similar on the whole were identified during the development of these habits. During the development of sensitization, the responses to test tactile stimulations and to applications of quinine and carrot juice appeared and/or were markedly intensified from 50 through 90 min from the moment of the first sensitizing stimulation. During the development of conditioning, responses to the tactile stimulations and quinine also appeared and/or intensified from 50-60 min, while responses to the conditional stimulus appeared and intensified approximately 30 min later (from 85-95 min). Phases of an initial marked increase, with a subsequent tendency to stabilization, in the level of Ca$^{2+}$ were observed in the command neurons during a brief period in the development of sensitization; during conditioning a temporary decrease was identified in the content of Ca$^{2+}$ in response to each combination of stimuli with its subsequent spontaneous decrease at 40-60 min. An increase in the level of Ca$^{2+}$, beginning during the development of sensitization from 50-60 min, and during conditioning from 85-90 min from the moment of the beginning of training, was observed in the long-term period. The protein synthesis blockers, anisomycin and cycloheximide, blocked the development of long-term neurophysiological and metabolic (Ca$^{2+}$) effects during the development of sensitization.

The study of the molecular—cellular bases of specific functions of the nervous system, and, in particular, of the mechanisms of learning and memory is one of the central problems of contemporary neurobiology. The "chemical" hypothesis of the integrative activity of the neuron, formulated by P. K. Anokhin from the prospectives of the theory of functional systems [1], has made a substantial contribution to the working out of this problem. According to this hypothesis, a genetically fixed, metabolic process, unfolding in the cytoplasm of the neuron, and specific in relation to specific synaptic structures may be the only possible pathway for the maintenance of the informational significance of excitations reaching the neuron for their subsequent integration.

At the present time experimental indications have appeared to the effect that, during the development of learning, synaptic excitations converging on the nerve cell induce not only change in the bioelectrical properties of the plasma membrane, but a complicated complex of membrane and cytoplasmic biochemical reactions as well [2, 3, 12, 14]. The results obtained have made it possible to ascertain a series of fundamental regularities and mechanisms of the processes of learning; however, as before, the tasks of identifying the "true" neurochemical correlates of learning and memory which are specific precisely for the formation of associative connection, and, consequently, the question of the intracellular mechanisms of the maintenance of the informational significance of excitations reaching the neuron, still remain urgent.

There are two principal hypotheses regarding the molecular nature of these mechanisms. According to the first hypothesis, information reaching the neuron is mediated by systems of second intracellular messengers. The role of the second messengers in the processes of learning has been studied quite actively; numerous data attesting to their involvement in the mechanisms of learning have been obtained [2, 12, 14, 15].
The other hypothesis is based on the fact that apparently a rather large number of synaptic inputs must have specific regulation in the "learning" neuron. Since the number of types of second messengers is fairly small, they can hardly pretend to the role of a specific marker of specific synaptic inputs; therefore it is postulated that the maintenance of the informational significance of excitations reaching the neuron is provided by protein molecules which are specific for the given synaptic inputs [2, 11]. According to this hypothesis, there is a selective "projection" of synaptic inputs to specific genetic loci of the neuron; direct and feedback connections with the genome provide for the selective genetic regulation of synaptic inputs, including during learning; however this hypothesis has primarily theoretical premises for its substantiation.

Progress in the understanding of the neuronal mechanisms of learning depends substantially on the use of promising experimental objects, behavior models, and new methods of molecular—cellular investigations. Molluscs, which have functionally and morphologically identified giant nerve cells which are readily accessible for various kinds of investigations are the classical objects utilized for the study of the problem under consideration; different types of associative and nonassociative learning are developed and maintained in them for a prolonged period fairly rapidly.

The method of intravital recording of the dynamics of calcium binding with hydrophobic intracellular substrates (Ca\textsubscript{b}) including the calcium-binding proteins, based on the use of the chlorotetracycline fluorescent probe is one of the appropriate contemporary research techniques [7, 10, 13]. This method makes it possible to evaluate the dynamics of the redistribution of Ca\textsubscript{b} in the identified neurons during learning.

On the basis of the above, the principal results of studies of recent years which we have carried out at the behavioral, neuronal, and molecular—cellular levels, to investigate the mechanisms which are specific for the development of the associative habit of food rejection in common snails are presented in the present paper. Since the sensitization accompanying them is an important component in the development of the defense habits, the effects obtained during the development of the associative habit were compared with the effects of "pure" sensitization, for the development of which the same stimulations were used as for reinforcement.

METHODS

The experiments were carried out in common snails, Helix lucorum. Sensitization or the associative defense habit of rejection of a specific type of food (carrots) were developed in the animals. Sensitization was developed in the behavioral experiments by the application of a 5–10% quinine solution to the head of the snail [4, 5]. The state of sensitization was evaluated on the basis of the duration of the defense reactions induced by the application of a weak (0.1%) quinine solution to the head. Testing was carried out every 15–20 min before and after the development of sensitization, as well as on the days following sensitization. In the case of the development of the associative habit [4], a 5–10% quinine solution which was applied to the snail's head while it attempted to eat carrots, was used as the reinforcing stimulus. One to five combined presentations of carrot and quinine were carried out every 15 min. The defense reaction to the presentation of carrot to the snail, as well as an increase in the latent periods of coming into contact with the food or refusal to eat, served as the criterion for the development of the conditioned connection. Testing of the maintenance of the habit was carried out 3–5 h after its development, as well as on the succeeding days following learning. In addition, the state of the sensitization accompanying learning was assessed in the snails following the method described above.

A semi-intact preparation of the animal was used for the neurophysiological and spectrophotometric investigations. The recording of the LP11 and RP11 cells, which belong to the group of command neurons of the defense behavior of common snails [4] was carried out by means of standard electrophysiological techniques.

Sensitization was developed by the application of a 5–10% quinine solution to the snail's head [5, 6]. Two to three subsequent applications of quinine were made every 15 min. The state of sensitization was tested with a 0.3% quinine solution, tactile stimulations, as well as by carrot juice which were applied to the snail's head every 15–20 min. The tactile stimulations were applied also to the middle portion of the foot and the mantle ridge.

During the development of learning, carrot juice (the conditional stimulus) was applied to the snail's lip for 30 sec. A 5–10% quinine solution was applied to the head immediately after the application of carrot juice. One to five combinations were carried out every 15 min. The test stimulations using carrot juice as well as the testing of the state of sensitization of the command neurons were carried out as during the development of sensitization.

To study the dynamics of Ca\textsubscript{b}, the microspectrofluorimetry of the LP11 and RP11 neurons stained with 50 μM chlorotetracycline was carried out using a LYUMAM KF luminescent microscope following the method previously described [6, 9, 10]. Sensitization and conditioning were developed as in electrophysiological experiments.

The participation of protein synthesis in the neuronal mechanisms of the development of sensitization was studied in neurophysiological and spectrophotometric experiments. Translation blockers, anisomycin or cycloheximide, in concentrations of 10–100 μM, were first introduced into the physiological solution bathing the CNS of the snails 1 h before the development of sensitization, and were then applied throughout the course of the entire experiment.

INVESTIGATION RESULTS

The application of a 0.1% quinine solution to the head of the intact snail induced defense reactions lasting 30–40 sec. The effect of a 5–10% quinine solution led to a generalized defense reaction of the molluscs lasting 4–6 min. Depending upon the features of the changes in the responses to the test stimulations by 0.1% quinine, the sensitized snails were divided into three groups [5]. An increase in the duration of defense reactions by 100–140% was identified in the snails of groups 1 and 2 immediately after the sensitizing stimula-