DIFFERENCES IN RESPONSES OF IDENTIFIED NEURONS TO CHEMOSTIMULI IN SATIATED AND HUNGRY GRAPE SNAILS

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In experiments on development of conditioned avoidance reflexes to food in the snail we observed that after learning the hungry animals (these reflexes did not develop in satiated snails) react to food in the same way as to an avoidance stimulus only on direct contact with the reinforced food form [3, 9]. In the majority of cases the behavior of hungry snails after learning was similar to that of satiated animals. Knowledge of the functional role of some neurons in the avoidance and feeding behavior of the snail [3] allowed us to carry out, using this object, comparative analysis of neuronal responses to chemostimulation in animals with differing levels of satiation both before and after learning, which will lead to a greater understanding of the role of the motivation factor in learning.

METHODS

For our experiments we used the lipchemoreceptor preparation where the connections between the chemosensitive peroral region (lip) and the periphraryngeal ring of ganglia is preserved (Fig. 1) and it is possible to apply a food stimulus inducing in the intact animal a food-procuring reaction (a drop of carrot juice), or a chemical stimulus inducing an avoidance reaction in the intact animal (quinine hydrochloride, \(10^{-2}\) M). The setting up of the bath (Fig. 1) offers the possibility of a separate flow of Ringer solution for cold-blooded animals through a chamber with the CNS and a chamber with the chemoreceptive structures.

The intracellular synchronous conduction away of the activity of 2-4 identified neurons took place by a standard method. Recording was by means of a pen recorder H-3021/4.

Taking into account that the edible snail can exist over a prolonged period without food [3], we regarded as hungry animals those that for at least 4-6 days were in an active state in a humid aquarium without food. No food residues were observed in the cut of such animals. We regarded as satiated those snails that were in an active state, had constant access to food, and whose cut contained food residues.

In this study we investigated a total of 67 animals, of whom 41 were hungry (98 neurons) and 26 satiated (54 neurons).

RESULTS

Before our investigation of the neuronal mechanisms forming the basis of a change in the animal behavior on satiation, we studied the behavior of hungry and satiated intact snails in behavioral experiments using standard methods [3]. We found that satiated animals refused...
food (avoided contact) in 96% of cases, while hungry snails commenced to eat the food in 87% of cases with a mean latent period of 6.8 ± 0.7 sec (n = 70). It follows from these data that it is not possible to develop avoidance conditioned reflexes (CR) to food in satiated animals. The behavior of satiated snails is very similar to that of hungry animals in which the avoidance CR to the given food form has been developed [9], which gives grounds for expecting some common features of the neuronal mechanisms important for the understanding of the learning mechanisms.

In the neurophysiological experiments using the lipchemoreceptor preparation (Fig. 1) we investigated the activity of integrative neurons of two forms of behavior of the snail, the avoidance behavior and food behavior. The selection of the cells was based on a knowledge of their functional characteristics. For the control of activity in the reflex arc of the avoidance reflex we selected giant neurons of the pleural (LPZ1) and parietal (LPa3, PPa3) ganglia, whose functional properties (for details see [3, 9]) allow these to be regarded as command neurons for avoidance behavior, since all the criteria proposed for describing the neurons as command neurons are fulfilled [8]. The important characteristic of the command neurons for interpretation of the results is the fact that the appearance in them of action potentials, induced by intracellular or adequate stimulation, always corresponds to an avoidance reaction by the animal.

We recorded the activity of the giant metacerebral neurons (LMtcl) which receive information from the chemoreceptive regions of the body surface of the animal and are modulators of the activity of motoneurons of feeding behavior [2].

Thus by recording the activity of the neurons described above on application of the chemostimulus we obtained information on the work of the reflex arcs of the feeding and avoidance behavior, it also being possible to assess the level of activation of the motor programs of the behavioral types mentioned.

On the lipchemoreceptor preparation of the hungry snail in 80% of cases no reaction in response to the application of a drop of juice was recorded in the command neurons of the avoidance behavior (Fig. 2), or a very weak reaction appeared in the form of subthreshold-stimulating postsynaptic potentials. In the giant metacerebral neuron recorded synchronously we always observed synaptic activation and spikes (Fig. 2) in response to the food stimulus. On recording of the activity of the same neurons on the preparation of a satiated animal there appeared, in response to the application of a drop of juice (in 70% of cases), a synaptic and spike reaction in command neurons of avoidance behavior (Fig. 3). The latent period of this reaction was 20-40 sec.

On application of a drop of quinine to the chemoreceptor region, in all cases we observed a reaction in both the command neurons of avoidance behavior and in the giant metacerebral cells, the reaction of the command neurons of the hungry snails always being weaker, however, than the reaction of the same neurons in satiated animals.

After development of the avoidance CR to food in intact animals or on the preparation in the neurophysiological experiment, a synaptic and spike activation (Fig. 4) with a long latent period, similar to that of the reaction of a satiated animal, is recorded in response to the drop of juice in the command neurons of the avoidance behavior. In some cases we observed in trained animals some activation of the pacemaker mechanism, but in the experiments in which we did not observe such activation we noted conditioned reactions. In control experiments performed using the method of pseudo-conditioning we observed no changes in the chemosensitivity of the skin receptors or in the reactions in the central neurons.

Thus on comparison of the responses of neurons of hungry and satiated animals to chemostimulation (carrot juice and quinine) we noted the appearance of responses to the juice in the command neurons of the avoidance behavior in satiated snails and an intensification of responses to quinine. Unfortunately, the extent of the response greatly varies from preparation to preparation, this posing difficulties in the quantitative assessment of the difference in the reactions of neurons of satiated and hungry animals. We therefore used an additional approach to this problem, which allowed us to model satiation directly in the neurophysiological experiment.

So far it is difficult to describe in detail the mechanisms of regulation of the degree of satiation of an animal, but the participation of both a humoral factor and a neuronal factor associated with it, i.e., of a change in the functional state and reaction capacity of neurons, is evident. In the experiments discussed above we have demonstrated the differences in