THE POSSIBLE ROLE OF MULTIPLICITY OF THE PACEMAKERS IN THE GIANT NEURONS OF INVERTEBRATES

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The rhythmic activity of neurons arises in a particular region which behaves as a special microfocus of excitation, a pacemaker. Recently, however, facts have been discovered (the presence of double potentials or of potentials of different amplitudes) which indicate that several pacemakers may operate within the same neuron [9, 2, 17, 10, 11, 13]. Double potentials were observed by Eccles [8] and P. G. Kostyuk [6] in motor neurons and by Phillips [15] in the pyramidal neurons of the cortex.

The object of the present investigation was to study the relationship between the electrical potentials of the pacemakers in the neurons of the hypopharyngeal ganglion of the grape snail and of the 6th abdominal ganglion of the crayfish in the conditions of primary and evoked pulse activity.

EXPERIMENTAL

Experiments were carried out on preparations of the hypopharyngeal ganglion of the grape snail (Helix pomatia L.) and the 6th abdominal ganglion of the crayfish (Astacus astacus). The potentials of the individual neurons were detected by means of glass microelectrodes, filled with a mixture of solutions of 2.5M KCl and 0.5M $Na_4Fe(CN)_6$, and the position of the point of the recording microelectrode was subsequently marked by a slightly modified Bultitude's method [12]. The pulse potentials were recorded on a "Diza" electromyograph.

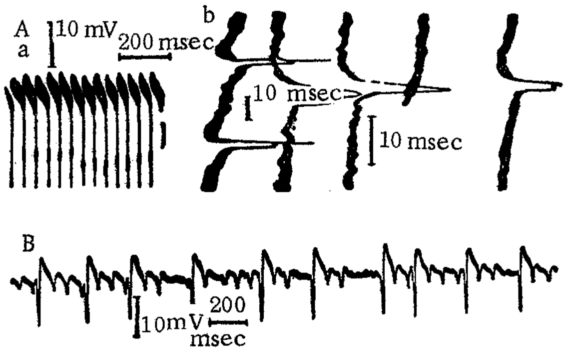

Fig. 1. Pulse potentials of a neuron of the 6th abdominal ganglion of a crayfish (A) and of the hypopharyngeal ganglion of a grape snail 6 h after dissection (B).
a) With a low scanning speed of the recording beam;
b) with a high speed.

DISCUSSION OF RESULTS

As the microelectrode was brought slowly up to the neuron generating an action potential, biphasic pulse potentials of low amplitude (200-400 μ V) began to be recorded while it was still some distance away (10-60μ). Further approximation of the microelectrode led either to an increase in the amplitude of the recorded potentials (up to several millivolts) followed by puncture of the membrane of the neuron and the recording of intracellular monophasic positive potentials of high amplitude (40-100 mV) or to cessation of the recording of potentials on account of the removal of the microelectrode from the "spontaneously" active neuron. From a consideration of the number of punctures of the membrane of the cells during each insertion of the microelectrode, the mean value of the extent of the zone of potential in the neuron (30 μ) which enables extracellular recording of potentials to be undertaken, and the

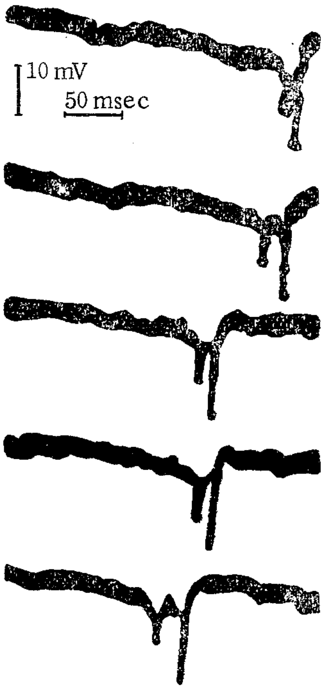

Fig. 2. Changes in electrical potentials of the pacemakers of a neuron in the 6th abdominal ganglion of the crayfish during slow movement of the intracellular microelectrode through a distance of 15 μ.

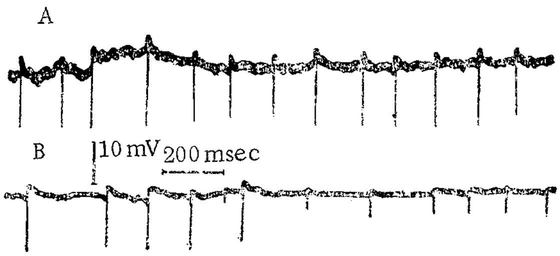

Fig. 3. Reaction of a neuron of the hypopharyngeal ganglion of a grape snail to electrical stimulation of the anal nerve. A) Background activity; B) pulse potentials 0.5 sec after the cessation of stimulation.

number of "spontaneously" active neurons recorded, the relative proportion of neurons possessing primary pulse activity could be be determined.

The experiments showed that one-tenth of the total number of neurons (mainly giant and large cells) of the hypopharyngeal ganglion of the grape snail and one-fourteenth of the neurons of the 6th abdominal ganglion of the crayfish possess primary pulse activity, i. e., they give discharges of potentials in the absence of special stimulation. In 86 experiments on the hypopharyngeal ganglion of the grape snail 938 neurons were discovered from which primary pulse activity could be recorded, and in 57 experiments on the 6th abdominal ganglion of the crayfish 456 "spontaneously" active neurons were discovered. In 15-20% of the neurons with primary pulse activity, when the point of the recording microelectrode was in a certain intracellular position pulse potentials were observed which had a notch at the level of 5-25 mV in in the rising phase or in the descending phase of the action potential spike (Fig. 1 A). It must be emphasized that this duplication of the potential was observed only during intracellular recording. In the course of prolonged observations (60 min or more) the shape of the recorded pulse potentials remained constant, and only in a few cases (when the functional state of the neuron deteriorated, as shown by a decrease in the amplitude and a change in the frequency of the action potentials) was the notch observed to become deeper and deeper, so that one component of the potential become separated from the other and the activity of two distinct pacemakers was recorded from one cell (fig. 1 B). Each pacemaker had its own amplitude and frequency characteristics, and these changed with time. Slight movement of the microelectrode (by a few microns) led to sharp changes in the pattern of the pulse activity: either the activity of one of the pacemakers disappeared, or their previously synchronous activity fell out of step (Fig. 2). It should be noted that, as analysis of the position of the recording microelectrodes showed, these potentials were usually typical of the large and giant neurons, with a body over 100 μ in diameter.

During electrical stimulation of one of the connectives of the ganglion, against the background of the primary pulse activity of the type mentioned above, responses of different types could be observed: an increase in the frequency of the first component accompanied by a decrease in the frequency or by the total inhibition of the second, or a decrease in the frequency of the first component accompanied by an increase in the frequency of the second (Fig. 3 A and B). At the same time it was found that subthreshold (for a change in the frequency of the spreading action potentials) stimuli led to a disturbance of the regular sequence of activity of the individual pacemakers. Consequently, besides the widely known mechanisms of reaction to stimulation in the form of a change in the membrane potential and in the frequency of the action potentials, the investigated neurons probably also possess an additional reactive mechanism in the form of a temporal redistribution of the electrical potentials of several pacemakers. A further increase in the strength of stimulation led to clear changes in the frequency of the action potentials.

The fact that a single intracellular microelectrode can record a duplicated spike potential or even 2 or 3 potentials of different amplitudes, and the change in the interspike intervals and also in the amplitudes of the potentials during displacement of the microelectrode inside the neuron demonstrate that the investigated cells contain two or more pacemakers, situated in different, but circumscribed parts of the cell body. The foregoing facts show that the space–time relationships of the electrical potentials of the pacemakers depend on the functional state of the particular neuron. The independent coexistence of several pacemakers in one neuron is possibly of definite biological significance. The evolution of the nervous system is known to have followed the path of an increase in the total number of its elements and an increase in the number of connections between them, as a result of the development of numerous ramifications of the dendrites [1, 7, 14, 16). This trend in the evolution of the nervous system has in fact led to the exceptionally high reliability of the construction of the brain of the higher vertebrates, responsible both for the partial duplication and the emergency organization, and for the statistical-probability method of arrangement of the elements in functional groups [3-5]. In the central nervous formations of the invertebrates the high reliability of function may perhaps be brought about in a special way as a result of the presence of several pacemakers in one neuron. This is possibly associated with the development of giant and large neurons in the ganglionic nervous system of the invertebrates, in which the partial duplication and the space–time summation of the influences of the different pacemakers in accordance with the laws of probability are responsible for the increase in the reliability of function.

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