AN INTRACELLULAR INVESTIGATION OF THE NEURONS OF THE CEREBRAL CORTEX OF THE UNANESTHETIZED RABBIT

(UDC 612.825.1-087)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 5,
pp. 3-7, May, 1965
Original article submitted August 9, 1964

The use of extracellular microelectrodes to investigate the activity of single neurons of the cerebral cortex of unanesthetized animals has recently become popular, for it enables not only the investigation of the activity of neurons in near-natural conditions [1, 14], but also the comparison of the neuronal activity and the behavioral reactions [15, 18], to be undertaken. On the other hand, considerably more information may be obtained by the use of intracellular methods of recording [5, 10, 12], which also have been used recently to investigate the cortical neurons [2, 3, 16, 17, 19-21]. However, intracellular experiments are performed either on animals under general anesthesia or on animals immobilized by means of operative procedures or, more rarely, by means of relaxants.

Our investigations have demonstrated the possibility of intracellular recording of the activity of the cortical neurons of unanesthetized rabbits. In this report we describe the technique of recording and the results of the investigation of the spontaneous activity of the cortical neurons.

EXPERIMENTAL METHOD

Experiments (21) were carried out on eight rabbits. A few days before the experiment the rabbit's skull was exposed under local or general anesthesia, over the sagittal and coronary sutures. Fixation of the rabbit while recordings were being made of the activity of its neurons was achieved by binding its limbs to a frame (in some experiments one or two limbs were left free). The rabbit's head as a rule was secured in a halter, although not tightly, so that the animal could still turn its head slightly. The technique does not call for the use of heavy stands and for complete immobilization of the animal.

The optic and sensorimotor areas of the cortex were investigated. In order to make recordings from the optic cortex, the point of maximal manifestation of the evoked potential on the occipital bone was selected [8]. To make recordings from the sensorimotor cortex, the region of representation of one of the limbs was determined by performing bipolar stimulation of the cortex through the preliminarily thinned bone (anteriorly to the coronary suture). By means of a dental drill, and under the control of a type MBS-1 microscope, a funnel-shaped hole was drilled in the skull with an upper diameter of the order of a few millimeters and a lower diameter of 0.5-1.5 mm. The small diameter of the hole in the skull ensured the maintenance of more physiological conditions and considerably reduced pulsation of the brain. In order to reduce pulsation still further, after insertion of the microelectrode the hole was flooded with agar-agar mixed with physiological saline, or with paraffin wax. Before insertion of the microelectrode, the dura was removed over the exposed area by means of fine hooks. The pia was usually left alone.

To introduce the microelectrode, an oil micromanipulator [5] was used, the delivering syringe of which was fixed with dental cement to the skull by means of a special support. By fixing the syringe with the microelectrode to the skull in this way the stability of the recording of the activity of the cell was ensured, despite the animal's movement. By means of the fine adjustment it was possible to move the microelectrode through a distance of 3 μ by turning the micrometer screw through one division. The coarse adjustment (30 μ to one division) was used when the microelectrode was brought to the surface of the cortex.

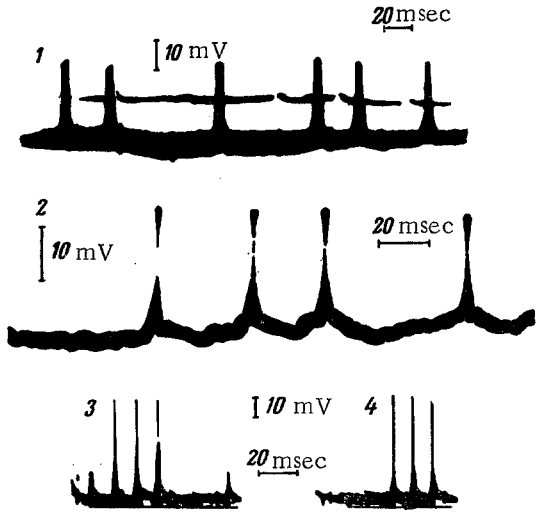

Fig. 1. Intracellular spontaneous discharges of neurons of the optic (1) and sensorimotor (2-4) areas of the cortex. The calibrating impulse marks 5 msec from the beginning of the time base. For explanation, see text.

The microelectrodes used for intracellular recording had a point with a diameter of less than 1 μ and a dc resistance of between 30 and 100 mΩ. The microelectrodes were filled with a 3 M solution of KCl or a 2 M solution of K^+ citrate.

The microelectrode was connected by means of a short, flexible silver wire to the grid lead of the input tube of a preamplifier with "negative capacitance," constructed in accordance with one of the variants of B. Ya. Pyatigorskii's scheme [9]. However, maximal compensation of the input capacitance was not used in all the experiments. The grid current of the input tube was maintained at a level below 10^{-11} A.

The potentials were recorded on the screen of a "Disa indicator" oscillograph and photographed on motion-picture film. As a parallel method, the signal could be fed for direct recording on a moving motion-picture film, and also into a type UBP 1-01 amplifier for acoustic control of the electrical activity and subsequent recording of the extracellular potentials.

After the experiment was finished the hole was flooded with agar-agar and paraffin wax. In most cases a new hole was made for the next experiment. Each rabbit was used in between 1 and 4 experiments.

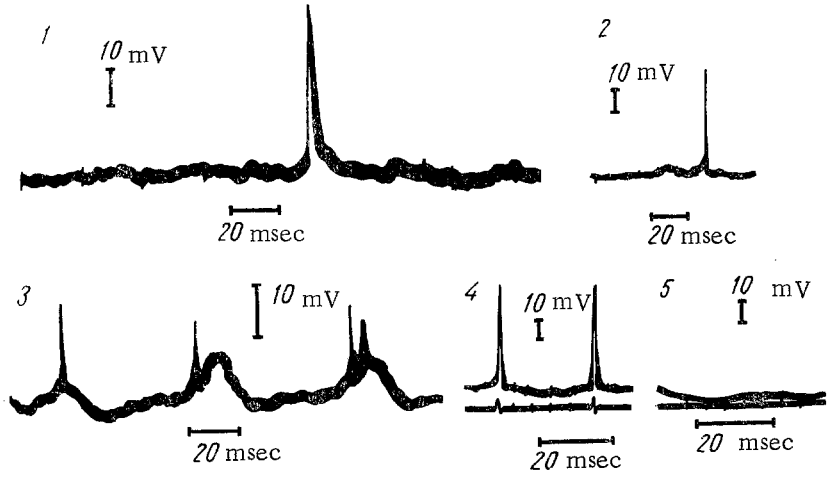

Fig. 2. Spontaneous synaptic potentials of neurons of the sensorimotor cortex. 1, 2) Continuous variations in level of membrane potential, causing discharge of the cell when the threshold level is reached; 3) large depolarization potentials causing cathodic depression of the mechanism of generation of the peak; 4) rhythmic spontaneous activity and 5) its cessation against the background of spontaneous depolarization of the cell. Tracing 5 was made 2-3 sec after tracing 4. On the lower beam (4) the signal was applied through a differential circuit with a differentiation constant of 10 msec.

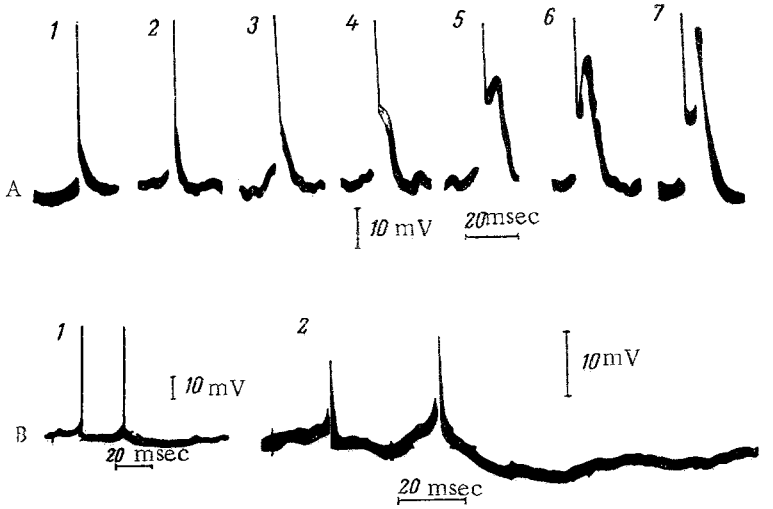

Fig. 3. After-potentials of neurons of the sensorimotor cortex. A) Depolarization after-potentials of the same neuron (1-4) and the change from after-depolarization to the second peak potential (5-7); B) hyperpolarization after-potentials of another neuron.

EXPERIMENTAL RESULTS

The activity of the neurons of the sensorimotor and optic areas of the cortex was recorded (Fig. 1). In most of the neurons penetrated by the microelectrode, clear signs of injury were observed (a high-frequency discharge), and their activity soon ceased. Either the activity of these neurons was not recorded or these recordings were excluded from the analysis. Relatively stable recordings were obtained from 11 neurons. The maximal time or recording of the intracellular activity was 15 min.

If the microelectrode was inserted to a depth of over 2 mm, i.e. its point lay in the white matter of the cortex, peaks were recorded without prepotentials of low amplitude (20-30 mV). The level of the membrane potential in these cases did not vary appreciably. Potentials such as these were evidently recorded from axons and were similar to the potentials which are assumed to arise in the axons of the spinal cord [6, 12] or of the cerebral cortex [16].

The most stable recordings were obtained in the sensorimotor cortex at the depth of 0.6-1.4 mm, corresponding approximately to layer V of the cortex [7, 22], in the upper part of which are situated the largest pyramidal neurons (with a mean length of 35 μ) [7]. The electrophysiological characteristics of the spontaneous activity of the neurons recorded in the sensorimotor cortex at this depth are described later.

The resting potentials (RP) of the investigated neurons had an amplitude of 47-87 mV. The most characteristic feature of the intracellular activity of these neurons was a constant variation in the level of the membrane potentials (Fig. 2, 1), suggesting a continuous subthreshold synaptic bombardment of the test neuron. This "synaptic noise" has previously been recorded in the motor neurons of the spinal cord [4, 10] and in the neurons of the cortex [16, 17], especially when the depth of anesthesia was slight [20]. However, in the present experiments on unanesthetized animals the continuous variation in the level of the membrane potential was a well marked and very constant phenomenon.

When the subthreshold variations were replaced by depolarization waves of larger amplitude—by excitatory postsynaptic potentials (EPSP),—the change from EPSP to action potential (AP) could be observed (see Fig. 1, 1, 2). The level of this change (the threshold) varied in different cells from 3 to 10 mV, but it was relatively constant for each neuron. The amplitude of the AP of the investigated neurons was 18-62 mV, usually about 50 mV. The maximal recorded margin by which the AP exceeded the RP was 10 mV. The duration of the AP at the half-amplitude level was 1.0-1.5 msec. Usually no indentation was observed in the ascending phase of the AP. Only during prolonged recording from a neuron was such an indentation found, and in some cases the 1st component of the AP could be distinguished (see Fig. 1, 3). This shows that in these cases, as in other types of neurons, the cell body is gradually enveloped by excitation, in two stages [11]. The developing AP usually had little effect on the further

development of the EPSP (see Fig. 1, 2 and Fig. 2, 1), as was found by other authors recording from the cortical neurons [2]. However, in some neurons the AP interrupted the development of the EPSP (see Fig. 2, 2), as is found in the case of monosynaptic excitation of the motor neurons of the spinal cord [11].

In most of the investigated neurons the peak potential was succeeded by a marked depolarization after-potential (see Fig. 2, 1 and Fig. 3, A), similar to the after potential of the neurons of the paleocortex [3]. The duration and amplitude of the depolarization after-potentials could differ in magnitude, not only in the AP of the different neurons, but also in the AP of the same neuron (see Fig. 3, A).

A hyperpolarization potential was recorded after the AP only occasionally, and in the same neuron it could be absent or it could be expressed to a varied degree (see Fig. 3, B), as was described by Phillips [19] when investigating the pyramidal neurons of the motor cortex of the cat. The impression was created that these hyperpolarization potentials are not true after-potentials, but inhibitory postsynaptic potentials, coming with a short delay after the EPSP evoking the AP. This raising of the level of the membrane potential (hyperpolarization), leading to the disappearance of the discharges from the cell, was also observed in the absence of a preceding EPSP and AP (see Fig. 2, 5). Meanwhile, the cessation of spontaneous activity was not always associated with appreciable hyperpolarization of the membrane (see Fig. 1, 3).

With an increase in the amplitude of the EPSP, cathodic depression of the second AP and absence of a discharge were observed when the threshold level of the neuron was exceeded by a considerable margin (see Fig. 2, 3). A phenomenon of this type has been reported in the cortical neurons of anesthetized animals in response to stimulation of nerve structures [20, 21] or in the region of an epileptic focus [13, 16, 23]. In the absence of general anesthesia it is evident that the powerful synaptic bombardment may give rise to cathodic inhibition even during normal activity of the cell.

The present study is only the first stage of the intracellular investigation of the cortical neurons of the waking rabbit. The results obtained show that the activity of the neurons of the unanesthetized animal possesses definite and specific features (a constant subthreshold synaptic bombardment, cathodic inhibition during spontaneous activity). These features may be of fundamental importance to the solution of problems in the general physiology of the nerve cell and also to the understanding of some of the aspects of the activity of the central nervous system.

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