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

(LDC 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 reac-
tions [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 inves-
tigation 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 record-
ings were being made of the activity of its neurons was achieved by binding its limbs to a frame (in some experi-
ments 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 perform-
ing 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.

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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.