MICROELECTRODE RECORDING OF RESPONSES IN VISUALLY OBSERVED NEURONS OF THE CAT MOTOR CORTEX

S. A. Evdokimov, M. O. Samoilov, and D. G. Semenov

With a contact optical system it is possible to carry out intravital studies of neurons and other structures, stained with vital dyes in reflected light in a specially prepared specimen of the cat cerebral cortex. The high-quality characteristics of the optical system used have made combined morphological and intracellular electrophysiological investigations of these neurons possible. The nature of intravital morphological changes in cortical neurons was established in response to their puncture by microelectrodes with tips with different external diameters and configurations; certain morpho-functional correlations were found in the response of pyramidal neurons to disturbance of their temperature regime.

INTRODUCTION

The physiological study of single cortical neurons of the mammalian brain by intracellular recording of their electrical activity is still attended by considerable difficulties. As a rule the worker does not know reliably from which cortical nerve cell the potentials are recorded. Pyramidal cells, whose axons formed the pyramidal tract [4–6], are exceptional in this respect. Methods of identification of neurons with the aid of various dyes injected electrophoretically into the cell are complicated and not sufficiently reliable. Moreover, even if a neuron is successfully tagged in this way, it is impossible to determine precisely where the microelectrode is located in the cell or to judge the precise morphological pattern of the neuron at the moment of recording its activity or of its electrical stimulation [2]. In 1968, Katsuki and coworkers [3] used their specially designed "dip-prism" microscope in order to study the electrical activity of single neurons of the cat motor cortex. However, the magnification and resolving power of this optical system were inadequate for use in combined morphoelectrophysiological studies.

EXPERIMENTAL METHOD

The writers suggest a basically different method of investigating mammalian cortical neurons. The anesthetized or immobilized animal (cats were mainly used) was secured in a stereotaxic apparatus. By minor changes in the design of the halter of this instrument, the animal's head could be moved in two horizontal planes.

A wide burr hole was drilled in the cranial bones in the frontal-parietal region. After opening of the dura, a U-shaped incision about 6–8 mm in depth was made at the required point of the cortex, allowing for the topography of the large pial blood vessels which supply most of the blood to the cortex. Since the pyramidal neurons of the motor cortex were the main objects of interest, this incision was usually made in the region of the anterior sigmoid and posterior sigmoid gyri. The area of cortex bounded by the U-shaped incision was undercut beneath the white matter and everted to occupy a certain position (Fig. 1a). Part of the area of cortex thus mobilized was then undercut at its base, in the plane α, the pia mater being preserved. As a result of this operation a preparation of the cortex, with adequate blood supply and preserving a considerable number of intracortical and cortico-subcortical nervous connections, and a free flap of cortex, preserving only its short intracortical nervous connections, were obtained.

Both in the attached and mobilized cortical preparations simultaneous investigations of neuronal and glial elements in different layers of the cortex can be undertaken. For microscopic examination and photography,
Fig. 1. Stages of operation to obtain attached (a) and mobilized (b) cortical preparations and alternative methods of aiming microelectrode at neuron under visual control (c, d). 1) Base of plume; 2) pia mater; 3) cortical preparation; 4) skin flap; 5) contact objective ×25; 6) neuron; 7) liquid in special bath; 8) surface of cortical preparation; 9) bent microelectrode.

The OI-ZO and OLK-2 (manufactured by LOMO) were used. These instruments were fixed to a special stand so that they could be moved in three planes. Incident polarized light and contact objectives were used for microscopy. Because of the high-quality optical characteristics of the objectives, the total magnification could reach 600. Without changing the position of the objective, brain tissues located at depths down to 60-150 μ (depending on the magnifications of the objectives) from the surface of the preparation could be examined by means of a special attachment without changing the position of the objective. In the investigation described below, a ×25 contact objective giving a depth of examination of down to 60 μ was chiefly used.

To detect nerve and glial cells in the cortex, low concentrations of the vital dye Methylene Blue (0.001-0.01%), pH 7.4, were applied to the surface of the cortical preparation.

Since a wide burr hole had to be drilled in order to carry out the dissection and to bring the objectives close to the preparation, it was most important to abolish brain pulsations due to pulsation of the blood vessels and to respiration. For this purpose the animal was fixed in a certain position and the cisterna magna opened, after which the removed cranial bones were replaced by a rigid plastic cover [1].

Measurements showed that the temperature of the surface layers of the preparation varied between 28 and 32°C (depending on the external environmental temperature). To keep the temperature of the preparation constant, a small bath was formed from the skin of the animal's head stretched over a transparent plastic framework. Solutions warmed to a certain temperature were passed into the bath through a closed system of tubes and a distributor. It is essential to note that, besides maintaining the temperature of the preparation constant, this system could also be used for direct application of various drugs to the neurons during examination, by injecting them into the solutions.

The electrophysiological part of the apparatus consisted of a micromanipulator with step motor designed to insert the micropipet in steps of 4 μ, a cathode follower connected to an amplifier, a stimulator with high-frequency attachment, and a bridge to neutralize the stimulating pulses of current at the input of the cathode follower. Potentials were recorded by means of a dual-beam cathode-ray oscilloscope. The microelectrodes were self-filling glass micropipets made from pyrex tubes with two or three capillary filaments. In some cases the electrodes were sharpened on a special quartz disc at an angle of about 30°. For work with the bath, specially curved microelectrodes were used. The micropipets were filled with 2 M KCl. Their resistance varied from 5 to 30 MΩ.

Insertion of the microelectrode into the cells of the preparation under visual control was carried out in several stages. First, the neuron to be studied was brought into the center of the visual field of the microscope by means of the combined moving stage and halter. Neurons whose nucleoli (the conventional center of the cell) were situated at a depth of 20-40 μ from the surface of the preparation were chosen. The objective was then raised above the preparation and the microelectrode positioned relative to the objective so that its tip coincided with the center of the visual field of the microscope and was at the focus of the objective.

The next stage of introduction of the microelectrode into the cell could be carried out in two ways. In the first method (Fig. 1c) the axis of the end part of the microelectrode was arranged perpendicularly to the axis of the objective; in the second method (Fig. 1d) the end part of the microelectrode was placed at a small angle to the frontal surface of the objective lens, so that the microelectrode tip was below the center of its axis, by a distance equal to the depth of the cell below the surface of the preparation. The microelectrode was then moved backward to the edge of the objective lens. In that position the microelectrode and objective were moved synchronously toward the preparation until the lens came into contact with