Electrical Responses of Astrocytic Glia from the Mammalian Central Nervous System Cultivated *in vitro*[1]

During the past years methods for the cultivation of various areas of the mammalian central nervous system have been developed[2]. In addition to neurons these explants provide large quantities of glial cells which become, in the course of cultivation, more or less isolated so that they can easily be observed with phase contrast microscopy. Time lapse cinematographic records revealed that such astrocytes can exhibit slow rhythmical movements[3] similar to those of amoebae or slime molds. For the present study, astrocytes from the midbrain and from the cerebellum of the kitten were used which were cultivated according to our standard methods on 12 × 50 mm cover slips in roller tubes. The cultures were kept in the incubator at 37°C for 30 to 50 days before they were used for electrophysiological experiments. By this time the astrocytes were separated from one another to such a degree that single elements could be easily distinguished (Fig. 1).

The cultures were mounted in a chamber approximately 4 mm deep. The bottom of this chamber consisted of a microscopic slide cut to approximately 12 mm in width and its roof was formed by the cover slip on which the cultures were grown. After the chamber was filled with Gey's balanced salt solution the cells were observed with Zeiss phase contrast optics at a magnification of approximately 600×, while the recording and stimulating electrodes were introduced from below through the open sides of the chamber at an angle of 10 to 30 degrees with the aid of a modified Leitz micromanipulator. The placement of the electrodes in and near the cells was made under visual control. The recording microelectrodes were filled with 3 *M* KCl-solution and had a tip diameter of less than 0·5 μ and a direct current resistance between 10 and 25 MΩ. The stimulating electrodes consisted of a pair of glass capillaries filled with Gey's balanced salt solution. The one close to the cell had a tip diameter between 5 and 15 μ, whereas the other one which was immersed some distance away in the chamber fluid was a plain glass tubing of about 1 mm in diameter.

Stimulating pulses were obtained from a Grass stimulator used in conjunction with an isolation unit. The pulse duration was approximately 1 ms. Intracellular potentials were recorded with a unity-gain preamplifier designed and constructed by BAK[4]. The input capacity of this amplifier was of the order of 0·3 pF and the grid current could be adjusted to zero at or near the working input voltage. A DuMont oscillograph was used for recording and the records were photographed with a Grass camera. In the attempt to pierce single astrocytes with the recording microelectrodes in many cases considerable difficulty was encountered due to the plasma clot in which the cells were embedded. The plasma clot made it difficult to achieve clean penetration of the cell body which, in such cases, was injured to such a degree that precise records could not be obtained. Similar difficulties were reported by CRAIN[5] in his experiments with chicken embryo spinal ganglia *in vitro*. Only in areas where clot liquefaction had taken place could clean penetrations of glia cell bodies be performed.

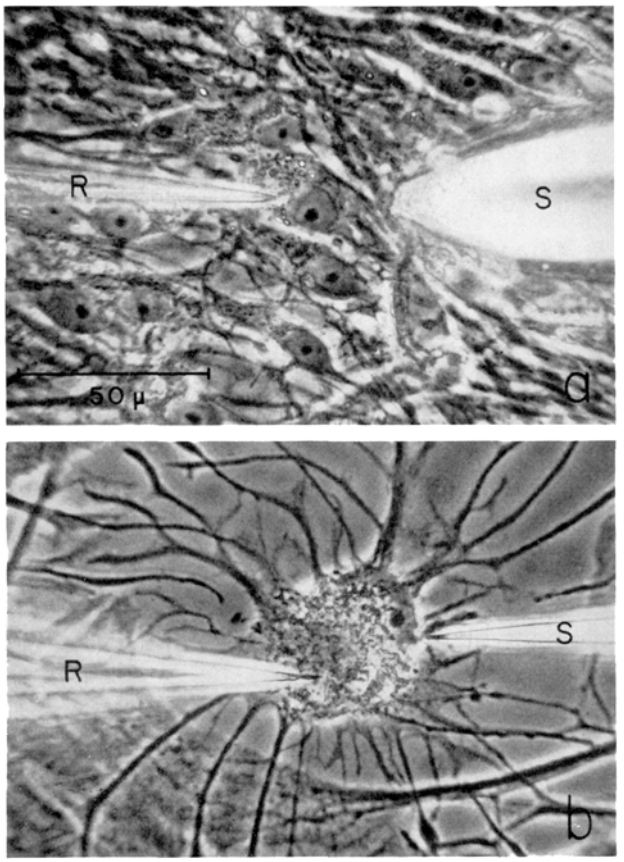

Fig. 1*a* and *b*. Photomicrographs showing representative astrocytes in the living state and the position of the electrodes during the recording time. *a* Field close to the center of the culture where the cells have retained their original netlike connections. A recording microelectrode (*R*) is introduced in one astrocyte whereas a larger stimulating electrode (S) is located near the cell. At times the stimulating electrode was used as an injection pipette by means of which a limited amount of isotonic KCl solution could be placed in the immediate vicinity of the cell. *b* A flatly spread out astrocyte somewhat isolated in the outer zone of outgrowth. The recording electrode (*R*) is introduced into the cell whereas a small stimulating electrode (*S*) is placed very close to the surface membrane of the cell. The outline of the nuclear membrane lies outside the plane of focus. Thirty-five day old cultures, phase contrast. The magnification is indicated by a bar in *a*.

On penetration of an astrocyte there was a sudden shift in the recorded direct current potential of usually less than 50 mV, the recording tip being negative to the potential of the surrounding fluid medium. On few occasions the potential shift was as large as 70 mV. When a small amount of an isotonic KCl solution was introduced by means of a small injection pipette placed in the immediate vicinity of the cell under investigation, the negativity of the intracellular potential decreased rapidly. On cessation of the outflow of the KCl solution from the injection pipette the negativity returned toward the original

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[3] W. HILD, Z. Zellforsch. *40*, 257 (1954).

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[5] S. M. CRAIN, J. comp. Neurol. *104*, 285 (1956).

level (Fig. 2, top). This observation indicates that the intracellular direct current potential of the astrocytes is similar to the resting potential of nerve or muscle fibers.

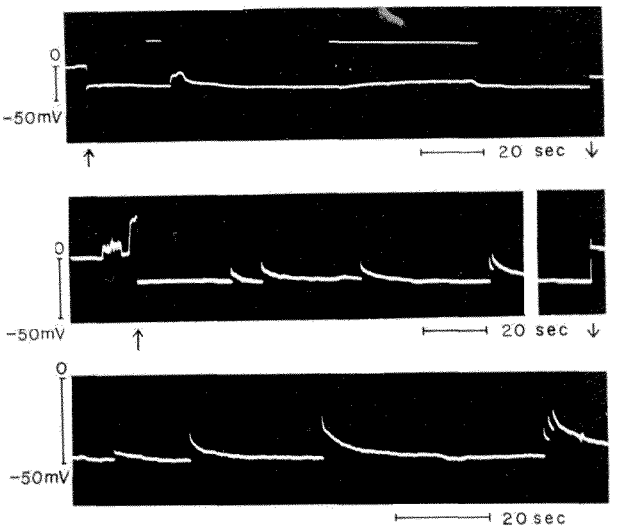

Fig. 2. – Electrical changes recorded from cultured glial cells on a continuously moving film. 0 is the potential level of the recording electrode when it is in the external fluid medium. The moment of impalement of the recording electrode into the cell is indicated by ↑, while that of removal from the cell by ↓. (Top) Shift of resting direct current level caused by the introduction of KCl solution into the immediate vicinity of the cell. Horizontal bars represent the KCl inflow; the first one was rather fast and the second quite slow. (Middle) Responses of glial cells to electrical stimuli. The shock intensities across the stimulating electrode with a resistance of 2 MΩ were (from left to right): – 50 V (showing almost no response), – 80 V, – 100 V, + 100 V, and + and – 100 V at 1 sec intervals. (Bottom) Similar responses to the shocks of: + 40 V, – 40 V, – 50 V, and three – 40 V at 1 s intervals. Note the difference in response magnitude to the cathodal and the anodal shocks of the same intensity and the summated responses to the rapidly repeated stimuli.

Application of electrical stimuli to impaled glial cells resulted in a transient reduction of their resting potential (Fig. 2, middle and bottom). No clear glial response was observed when the resting potential was less than 15–20 mV. The degree of this potential reduction increased with the strength of the applied electric shock. The time course of the potential change was roughly exponential, the $1/e$ decay time being approximately 4 s at about 27°C. The time course was about one thousand times as long as that of the electrical response of neurons in the same culture. Unless the stimulus strength exceeded an injurious high level, the time course of the potential change was independent of the stimulus strength. With a relatively small stimulating electrode placed close to the surface of the astrocytes (Fig. 1*b*), a cathodal stimulus was more effective than an anodal stimulus of the same strength. When a stimulating cathode with a tip opening of about 10 μ in diameter was placed at a distance of about 15 μ from the surface of the cell (Fig. 1*a*), a distinct effect of stimulation could be observed at current intensities greater than 10–20 μA.

The effectiveness of stimulating pulses applied to an astrocyte was found to increase gradually when the stimulus duration was increased from 1 m/s to 20 m/s. For stimulus durations shorter than about 0·2 m/s, the effectiveness decreased rapidly with shorter durations. A second or more stimuli following the first one after a short interval evoked a response superimposed on the falling phase of the preceeding response (Fig. 2, middle and bottom). All these properties of the potential variation in the surface membrane of the glial cell resembled the electric response' of the slime mold, *Physarum polycephalum*, which was investigated by TASAKI and KAMIYA[6].

There was no significant difference in the responses of various modulatory types of astrocytes as they are known to exist in tissue culture material. Almost completely isolated cell forms, which had flattened out to a considerable degree (Fig. 1*b*), showed the same responses as astrocytic elements which had remained in contact with each other as in the more central parts of the culture (Fig. 1*a*).

The resting potential of many cells remained uninfluenced after completely replacing the surrounding fluid medium with a cocaine (0·05–0·1%) or ethyl-urethane (1·5–2%) salt solution. On several occasions a strong but reversible suppression of the 'response' of the glia cells was demonstrated with these narcotics. Our present interpretation of the 'glia response' is the following: The surface membrane of the astrocyte is capable of developing an electrical response by a mechanism similar to that of the nerve or muscle fiber membrane. The interaction, however, between the 'responding' and the resting area of the membrane is not strong enough to cause re-stimulation of the resting area by the electric current arising from the responding area. An increase in the stimulus strength increases the amplitude of the observed response by decreasing the membrane area remaining unexcited by the stimulus. The low resistivity of the surface membrane appears to be the cause for the impossibility of re-stimulation.

Since astrocytes constitute a very large percentage of the entire cell population in the mammalian central nervous system, the finding that these cells can develop extremely slow electric responses may be of significance in the interpretation of slow time scale recordings taken from the intact surface of the brain. It appears important, therefore, to examine the relationship between the slow electric phenomena in the cortex, such as spreading depression, and the response of the glial cells.

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Zusammenfassung

Einzelne Astrozyten in Gewebekulturen vom Klein- und Mittelhirn junger Katzen wurden elektrophysiologisch untersucht. Ableitungen mit Mikroelektroden, die unter mikroskopischer Kontrolle in die Gliazellen eingestochen waren, zeigten Ruhepotentiale ähnlich den aus Nerven- und Muskelfasern bekannten. Durch isolierte Umspülung der Gliazellen mit isotonischer KCl-Lösung konnten diese Ruhepotentiale aufgehoben werden. Elektrische Reizung der Gliazellen verursachte eine temporäre Reduktion des Ruhepotentials für 4–5 s. Es werden Angaben gemacht über die Reaktion der Gliazellen nach Kokainisierung und nach Urethannarkose.

[6] I. TASAKI and N. KAMIYA, Protoplasma *39*, 333 (1950).

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