A MULTIELECTRODE PERFUSION CHAMBER FOR TISSUE CULTURE RESEARCH

M. B. Shtark, A. S. Ratushnyak,
L. V. Voskresenskaya, and S. N. Olenev

UDC 57.085.23

A chamber for neurophysiological investigations of nerve-tissue cultures, consisting essentially of a 12-electrode matrix made by spraying metal on a slide or coverslip, is described. Experiments involving recording electrical activity, stimulation, and directing the growth of a nerve-tissue culture can be carried out in such a chamber. Because of the design of the chamber the location of the electrode in the culture can be verified visually at the same time.

KEY WORDS: nerve tissue culture; bioelectrical activity.

The extensive use of nerve-tissue cultures at the present time in electrophysiological research is based on the use of known macroelectrode and microelectrode methods [4, 5, 9-11, 14, 15]. These methods naturally allow the electrical activity of the individual cells and their aggregations to be recorded chiefly under acute experimental conditions but with disturbance of sterility. Some progress in this respect was made by the recent work of Crain [12], who describes a multielectrode chamber consisting of 6 liquid microelectrodes controlled by magnetically operated knobs. Clearly these methods, together with others at present suggested for the same experimental purposes [8], notwithstanding their originality, are nevertheless based on the use of the principles of mechanical movement of microelectrodes oriented relative to the sources of the biopotentials.

Modern methods of microelectrode technology and vacuum engineering have provided the solution to many electrophysiological problems of nerve tissue culture work by what is fundamentally a different method [1]. Methods of vacuum spraying with photographic templates or of etching through an acid-resistant photosensitive layer [2] can be used to obtain special multielectrode matrices of nontoxic metals providing electrical contact with cells at any point of a tissue culture. This paper describes the construction and use of an apparatus of this type.

A. The method of making the multielectrode matrix consists of two stages: making the metal masks and spraying the conducting and insulating layers. The masks are made by a photolithographic method. The pattern of the spaces in them corresponds to the essential pattern of conducting strips and insulating coverings. The mask is placed on the glass base after which it is sprayed with the basic metal (usually titanium) in vacuo and then covered with gold or platinum. After these operations the mask is put in position to cover the places on the conducting strips designed for the formation of the contact areas and the insulating layer (silica) is then sprayed on. Two areas not covered with insulation are formed on each conducting strip. One of them forms the active tip of the electrode itself, the other an area for connection to the output of an amplifier, stimulator, and so on.

B. Technique of culture and electrophysiological investigation. The method is based on Bornstein's "flying slide" method [9]. Pieces of embryonic (18-19 days of prenatal ontogeny) and postnatal (3 days)

Laboratory of Neurophysiological Mechanisms of Adaptation, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR. Institute of Automation and Electrometry, Siberian Division, Academy of Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 9, pp. 122-124, September, 1974. Original article submitted June 20, 1973.

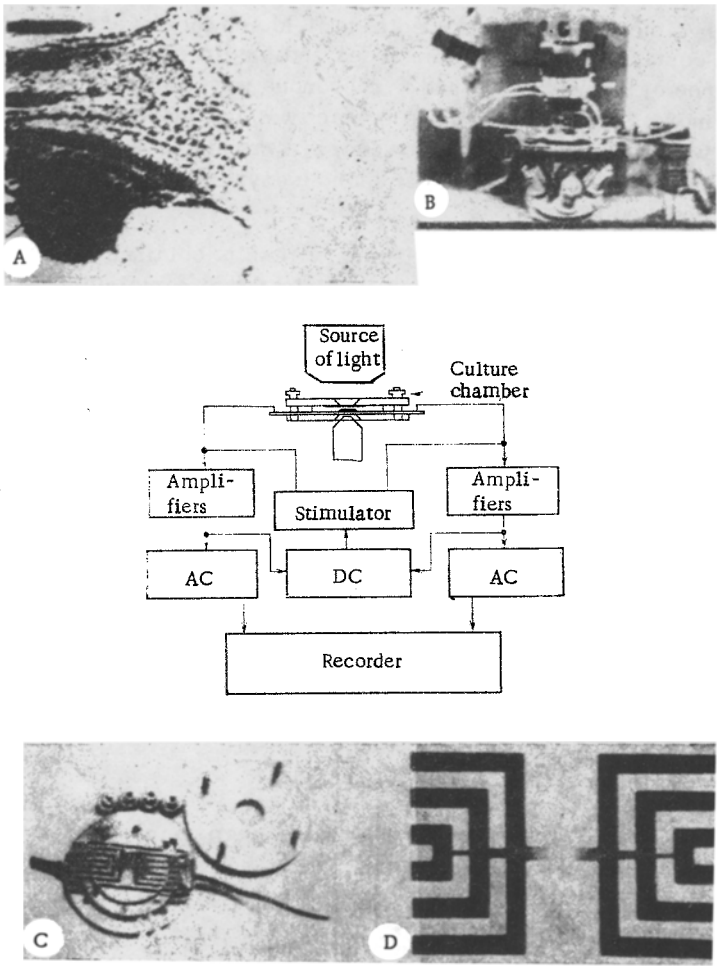

Fig. 1

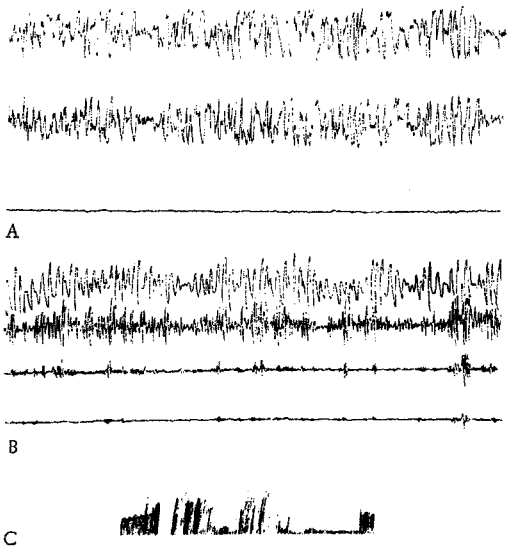

Fig. 2

Fig. 1. Multielectrode chamber for tissue culture and recording electrical activity: A) explant on electrodes; B) general view of chamber in thermostat of MBI-13 microscope; C, D) chamber with matrix electrodes. In the center – block diagram of the system for recording and processing electrical activity and for stimulating culture from a computer. AC) analog computer; DC) digital computer. Type USCh-1-03 recorder.

Fig. 2. Electrophysiological analysis of tissue culture activity: A) integral electrical activity of explant shown in Fig. 1A: last curve recorded with electrode outside explant; B) frequency analysis (from top to bottom: δ, θ, α, β); C) integration of δ-rhythm on analog computer. Rat aged 3 days, 12th day of culture.

brain tissue were explanted on the matrix-electrodes so that explants measuring not more than 0.5-0.8 mm were fixed in the zone of the electrode fields (Fig. 1A). The matrix electrodes were fixed in a perfusion chamber and placed on a constant-temperature stage of an inverted microscope of the MBI-13 type (Fig. 1B-D). Perfusion with a medium, the composition of which was described earlier [7], was carried out with the aid of communicating vessels. Without disturbance of sterility, the medium in the perfusion chamber was changed periodically at intervals of 2-3 days.

The widened bases of the sprayed focal microelectrodes (the tip did not exceed 30 nm) were connected with the inputs of the amplifiers. Integral electrical activity was estimated in real time with the aid of MN-7 analog computers, as described previously [3]. By the use of this system it was possible to record the electrical activity of nerve tissue cultures growing on the matrix electrodes for a comparatively long time.

Integral electrical activity of the EEG type (Fig. 2) can be recorded at intervals of 5-7 to 25-30 days of cultivation. The character of the activity, its frequency spectrum, and the amplitude of the waves were

evidently determined by two factors: the nearness of the explant to the electrode field and the "age" of the culture. During bipolar recording and with direct contact between the electrodes and the growing explants, electrical activity was characterized by the presence of asynchronous and synchronous waves in the α-, β-, and Θ-bands (Fig. 2) with an amplitude of not more than 500-700 μV. If contact was not made between the explant and the electrodes, an isoelectric line was recorded (Fig. 2); however, it was also observed more often than not during the investigation of explants cultivated for longer than 30 days. With electrodes close together, synchronous activity was usually recorded (Fig. 2).

The only purpose of this paper is to describe the method and the preliminary results obtained by its use for electrophysiological analysis of nerve tissue cultures. Nevertheless it is clear that completely new conditions for the study of nerve tissue cultures can be created on the basis of this principle. For a start, prolonged observations on the activity of nerve tissue cultures can be made and the electrical characteristics compared with certain other functional parameters (movement, for example). The use of long-term multielectrode perfusion chambers enables growth of the tissue culture to be controlled through the creation of electric fields of varied configuration. With the use of more complex structures (patterns) of electrode matrix, this control of growth and stimulation can be computerized (Fig. 1). This method would seem to offer many interesting new prospects for tissue culture research.

LITERATURE CITED

1. V. K. Bykhovskii, M. B. Shtark, and S. P. Shurin, in: The Automation of Scientific Research on the Basis of Application [in Russian], Novosibirsk (1970), p. 43.
2. N. K. Ivanov-Esipovich, Microcircuit Technology [in Russian], Moscow (1972).
3. V. P. Kaznacheev, M. B. Shtark, V. P. Leutin, et al., Dokl. Akad. Nauk SSSR, 202, No. 4, 978 (1972).
4. I. N. Kokina and N. M. Zhukovskaya, Tsitologiya, No. 8, 995 (1968).
5. V. F. Bystrov, L. V. Voskresenskaya, et al., Tsitologiya, No. 6, 122 (1970).
6. N. A. Smirnova and V. E. Shungskaya, Uspekhi Fiziol. Nauk, No. 3, 3 (1970).
7. M. B. Shtark, L. V. Voskresenskaya, A. S. Ratushnyak, et al., Dokl. Akad. Nauk SSSR, 202, No. 3, 734 (1972).
8. V. M. Yachin, A. M. Khokhlov, V. I. Reshetnikov, et al., Tsitologiya, No. 7, 918 (1972).
9. M. Bornstein, Lab. Invest., 7, 134 (1958).
10. S. Crain, Anat. Rec., 151, 340 (1965).
11. S. Crain, M. Bornstein, and E. Peterson, Brain Res., 8, 363 (1968).
12. S. Crain, J. Appl. Physiol., 31, 926 (1971).
13. S. Crain and M. Cornes, J. Neurobiol., 3, 25 (1972).
14. V. Schlapfer, in: Bioelectric Activity of Neurons in Tissue Culture, Berkeley, Calif. (1969).
15. B. S. Scott, V. E. Engeldert, and R. C. Eisher, Exp. Neurol., 23, 230 (1969).
16. L. V. Voskresenskaya, A. S. Ratushnyak, and M. B. Shtark (M. B. Stark), Proc. Internat. Union Physiol. Sci., 9, 1880 (1971).