The effect of pulsating electrical current on isolated nerve cells from the chick cerebral hemispheres cultivated in the rose chamber

G. G. Jaros[1], M. Sensenbrenner[2] and P. Mandel

Centre de Neurochimie du CNRS, 11, rue Humann, F–67085 Strasbourg Cédex (France), 7 July 1976

Summary. It was shown that 73% of the fusiform cells obtained in short-term cultures of dissociated cerebral hemispheres of the chick embryo become oriented under the effect of a pulsating current, applied during the first 24 h of culture.

Various authors investigated the effects of electric current on nerve cells in culture during the years 1920 to 1946, obtaining a variety of results. 3 of the groups found orientation of nerve fibres due to the passage of a direct electric current through their cultures[3–5]. Other groups reported that the current had no effect at all[6,7], while Williams[8] found total destruction of the cells on exposure to the current. Only Peterfi and Williams[9,10] varied the current intensity sufficiently to obtain all the above-mentioned effects. These authors found that when the current was weak and the exposure to current short, the cells were unaffected. However, increase in current strength and exposure time caused the appearance of granules in the cytoplasm, followed by cytoplasmic movement along the direction of current flow. At high current intensities, vacuoles appeared in the cytoplasm and the cells, and the cells degenerated.

In view of the conflicting results and the long period of inactivity in this field of research, it was suggested that the problem should be re-examined[11,12]. We have studied the effect of both direct and pulsating current on developing dissociated cerebral hemisphere cells from the chick embryo. The experiments with direct current have been reported elsewhere[13]. In the present communication we report the effect of pulsating square-wave current on dissociated nerve cells.

Materials and methods. Dissociated cerebral hemisphere cells from 7-day-old chick embryos were cultivated in Rose chambers on a collagen substrate, according to the methods described previously[14–16]. The nutrient medium consisted of Eagle's minimal medium, 20% fetal calf serum and 500 mg % glucose. The cells were kept isolated and the proliferation of the glial cells was depressed by the method described elsewhere[17]. The electrode system consisted of 2 gold electrode plates constructured as follows: each of the 2 electrodes was in form of a strip of gold

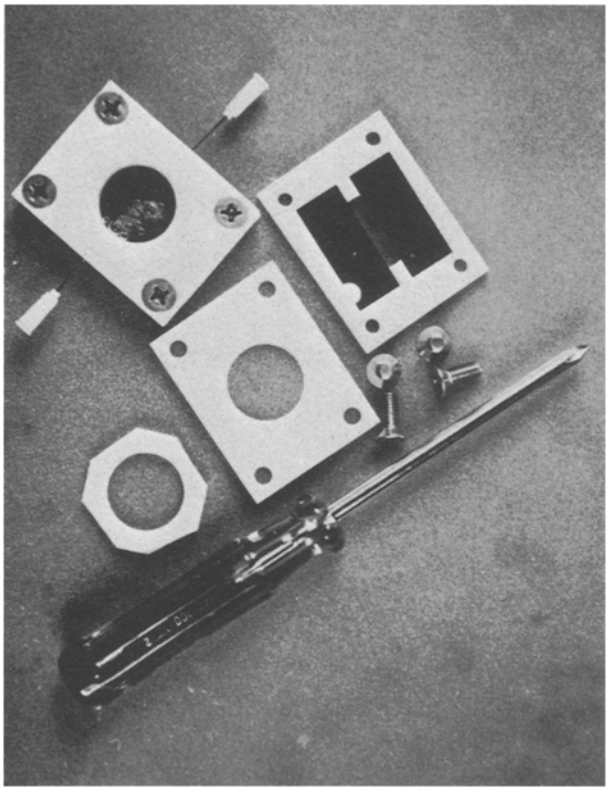

Fig. 1. The Rose Chamber assembly.

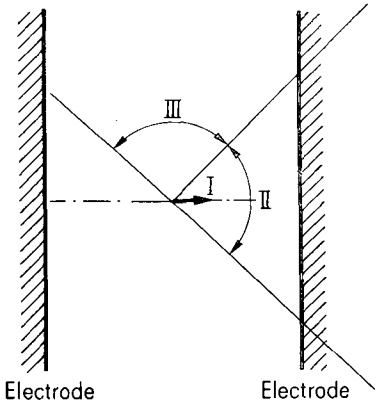

Fig. 2. The direction (I) and the directional ranges (II and III) in which the cells were counted.

1 Present address: Department of Physiology, University of Pretoria, Pretoria 0002, South Africa.
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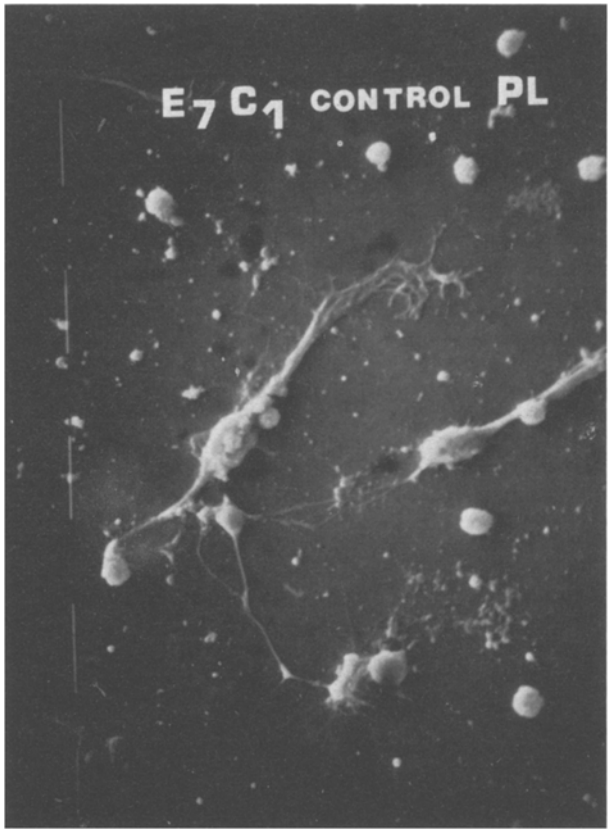

Fig. 3. An example of the fusiform cells the orientation of which was studied. E_7 = 7-day-old embryo, C_1 = 1 day in culture. Length of marker line = 10 µm.

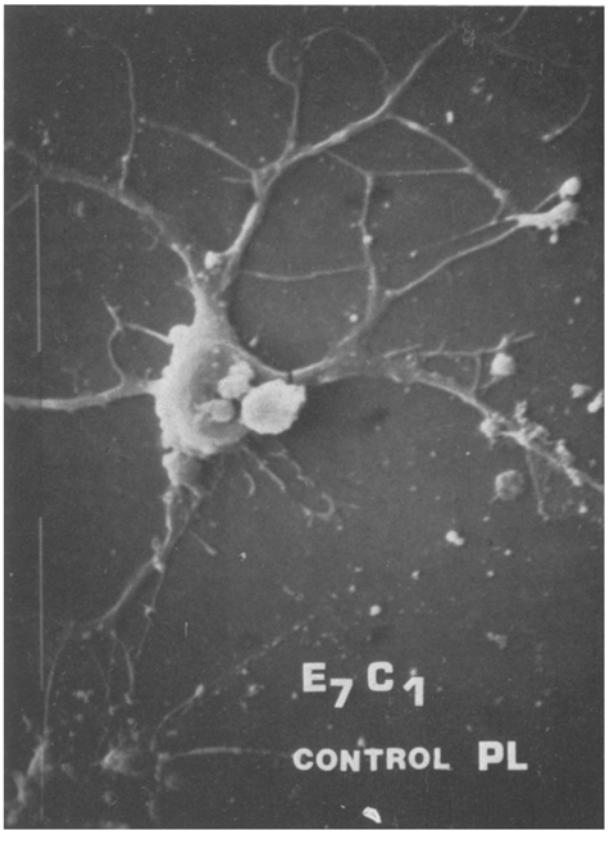

Fig. 4. An example of a multipolar cell. E_7 = 7-day-old embryo, C_1 = 1 day in culture. Length of marker line = 10 µm.

(15 × 40 mm) evaporated onto the coverslip (40 × 40 mm) that was to serve as the bottom coverslip in the Rose chamber assembly (figure 1). There was a 5 mm wide strip of the coverslip surface left non-covered by the gold between the 2 electrode strips. The cells grew on this strip that was prepared for cultivation by using collagen substrate. The current passed between the 2 electrodes was pulsating squarewave current provided by a physiological stimulator of low output impedance. The frequency of the current was 1 Hz, the pulse duration 1 msec and the intensity of the current varied between 50 and 300 µA/mm². This range of current intensity was chosen on the basis of preliminary experiments, showing that intensities below 50 µA/mm² were ineffective and those above 300 µA/mm² destructive in a way similar to that reported earlier[13]. The exposure to the current lasted for 24 h, starting at 0, 1 and 2 days of culture time. About 150 cultures were prepared including the preliminary experiments. The cultures were observed by phase contrast microscopy after 1, 2 and 4 days of incubation. Orientation was determined by counting the number of cells having their longitudinal axes in predetermined directions or directional ranges (figure 2). These included the direction joining the electrodes, the 90° range with 45° on either side of the line joining the electrodes, and the 90° range with 45° on either side of the perpendicular to the line joining the electrodes. Control cultures were prepared in identical chambers and under identical conditions for each experiment.

Results. Isolated neurons from dissociated cerebral hemispheres of the 7-day-old chick embryo develop on a collagen substrate in culture as follows[17]. The cells settle on the substrate after about 2–3 h and start to grow processes at about 16 h in culture. First the cells are bipolar (figure 3) and later become multipolar (figure 4). When the current was passed starting at the time of culture, an orientation was observed after 24 h in culture demonstrating that 72.9% of the cells had their axes at an angle less than 45° with the line joining the electrodes and 27.1% of the cells more than 45°. Of the cells in the first group about 58% took up positions with their longitudinal axes in the same direction as the line joining the electrodes. Only about 2% of the total cells were found lying perpendicular to the above direction. As most of the cells transformed into multipolar cells after about 2 days in culture, no further orientation was observed at this stage. Similarly there was no orientation in any of the control cultures.

Discussion. From the above results, it is clear that, although the electrical current does not cause complete orientation of the bipolar cells, it certainly influences it to a great extent. The effect is on the orientation of the longitudinal axis of the cell body and not in the direction of the neurites. In fact when multipolar differentiation begins to take place, the cellular processes lie in all directions. This seems to indicate that the electric current influences the direction of movement of the fusiform cells. The exact mechanism still needs to be elucidated.