A Technique for Marking the Site of Recording with Capillary Micro-electrodes

By K. H. BULTITUDE

(From the Department of Zoology and Comparative Physiology, The University of Birmingham)

With one plate (fig. 1)

Summary

Records were made from the hind-brain of 15-cm tench (*Tinca tinca*, Osteichthyes) by the use of capillary micro-electrodes. At the completion of recording the electrodes were left in position and the site of recording was marked by the following method. The Ringer bathing the brain was replaced by a mixture of equal parts of 110 mM $FeCl_3$ and Ringer. After 4 h, ferrocyanide ions were released electrolytically from the micro-electrode. The electrolyte in the micro-electrodes was 2·5 M KCl+0·5 M $Na_4Fe(CN)_6$. The optimum quantity of electricity for the electrolysis was found to be 2×10^{-4} coulombs. The brain was then fixed overnight in 90% alcohol, sectioned at 20μ, and stained with eosin.

Precipitates of Prussian blue about 20μ in diameter were found at the site of the electrode tip.

Introduction

DURING an attempt to record activity from the hind-brain of 15-cm tench (*Tinca tinca*, Osteichthyes) with small diameter capillary electrodes, the need became apparent for a marking technique to permit accurate localization of the site of recording. The brain-tissue in lower vertebrates is too soft for micro-electrodes to leave tracks (Woldrings and Dirken, 1951). Further, if it were possible to cause the micro-electrodes to leave tracks, it is unlikely that precise localization of the tip region would be possible; nor is it possible with capillary micro-electrodes to use the accepted techniques for marking metal electrodes: release of metallic ions (Scheibel and Scheibel, 1956), or lesions produced by burning.

The ideal marking technique would have to satisfy several requirements. It should be simple to operate and not liable to cause movement of the electrode relative to the brain. The mark made should be distinct and specific to the site of the electrode tip. It was thought that the electrolytic release of ferrocyanide ions from the electrode and their precipitation as Prussian blue by a solution of ferric ions bathing the brain would meet these requirements. Subsequent histological investigation would permit accurate localization of the Prussian blue mark.

If the electrolyte in the micro-electrode contained ferrocyanide ions, the only procedure necessary would be to exchange the recording leads for leads from a convenient d.c. supply, and to add a ferric salt to the Ringer bathing the preparation. Neither of these operations would be liable to disturb the site

Quarterly Journal of Microscopical Science, Vol. 99, part 1, pp. 61–63, Jan. 1958.]

of the electrode tip and the mark produced would be permanent, distinct, specific, and readily identifiable.

Procedure

The micro-electrodes, made with a two-phase, vertical-pull machine (Winsbury, 1956) from 3-mm soft glass tubing, were filled with an electrolyte of the following composition: 2·5 M KCl+0·5 M $Na_4Fe(CN)_6$ (Tasaki, Polley, and Orrego (1954)). Electrodes with an impedance of less than 3 or more than 35 megohms were rejected.

At the end of recording the electrode was left in position and the Ringer bathing the preparation was replaced by a saline solution containing ferric salts and left for not less than 4 h. The most satisfactory solution was found to be a mixture of equal parts of 110 mM ferric chloride and Ringer. This was made up immediately before use from a 1·1 M solution of ferric chloride, as in dilute solution ferric chloride rapidly hydrolyses to colloidal ferric hydroxide, which diffuses very slowly. The preparation was allowed to stand in this solution for not less than 4 h. When this was inconvenient it was found possible to reduce loss of histological detail by transferring the preparation and electrode, clamped together, to a refrigerator until it was convenient to carry out the rest of the marking procedure.

A minimum of 4 h was found necessary in order that the ferric ions could diffuse throughout the brain (distances up to 4 mm in this case). It was sometimes found that the slight fixing action of ferric chloride was sufficient to leave a conspicuous electrode track, but this was insufficiently reliable to be used by itself for site recognition. This is because tracks were not frequently found and, if present, could rarely be followed to the electrode tip; in some preparations they were readily confused with other artifacts produced in the brain by subsequent histological treatment.

At the end of the diffusion period, ferrocyanide ions were released electrolytically from the electrode. This was done by applying a potential difference of 4-V from a battery between inside (cathode) and outside (anode) of the micro-electrode. The optimum quantity of electricity was found to be 2×10^{-4} coulombs, i.e. with a 30 megohm micro-electrode the 4-V potential difference was applied for 30 min (current = 0·13 μA for 1,800 sec) and with a 10 megohm micro-electrode this quantity was supplied in 10 min (0·4 μA for 600 sec). The minimum quantity of electricity necessary was about 1×10^{-4} coulombs, but the use of twice this amount was found to give a more readily identifiable mark with greater consistency. The mark produced was not large enough to impair the required degree of accuracy and the use of 2×10^{-4}

FIG. 1 (plate). Photomicrographs of 20-μ transverse sections of tench hind-brain stained with eosin, showing the Prussian blue marks produced at the tip of micro-electrodes.

A, mid-facial lobe. There is a slight electrode tract and fixation artifact. 3×10^{-4} coulombs were used in electrolysis.

B, entry of spinal V-root. The mark at the tip of the electrode is clear but there has been some diffusion of ferrocyanide away from this region. 2×10^{-4} coulombs were used in electrolysis.

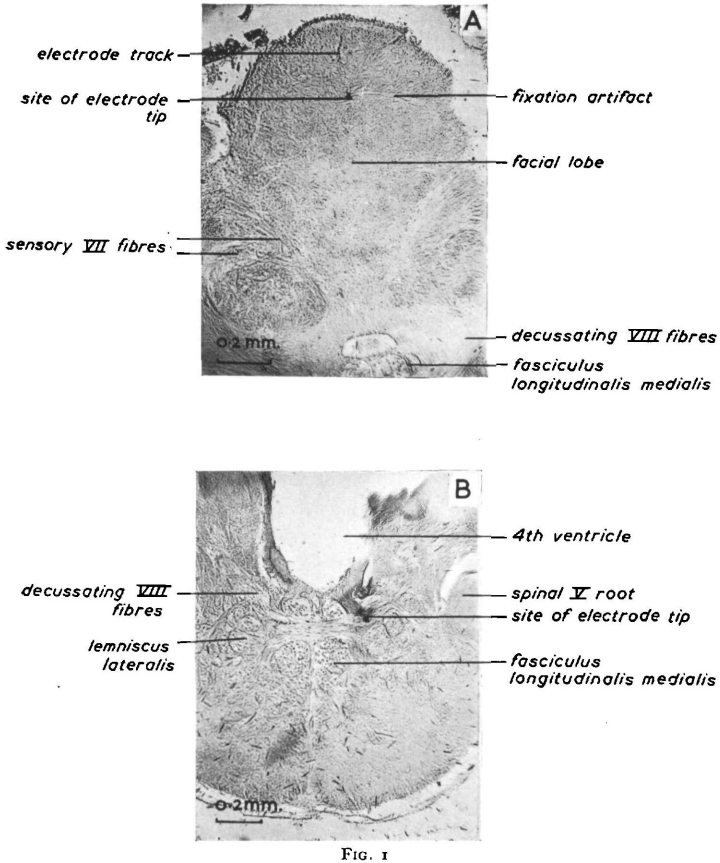

FIG. 1

K. H. BULTIDUDE

coulombs also reduced the possibility of failure due to an undetected partial blocking of the electrode.

To prevent the diffusion of ferrocyanide ions away from the electrode tip it was found necessary to wait for complete diffusion of the ferric ions through the brain before starting the electrolysis. If this precaution was not observed, the marks were absent or a diffuse precipitate was formed.

The brain was fixed in 90% alcohol immediately after electrolysis. The optimum time for fixation was found to be about 15 h, i.e. overnight. The brains were then blocked in 58° C paraffin wax and serial transverse sections were cut at 20 μ through the relevant region. These were then stained lightly with eosin and mounted in DePeX.

Results

Use of this technique has consistently produced a mark at the site of the electrode tip and the sections were found to show sufficient histological detail for the accurate identification of this site (fig. 1). The nervous elements stain in different intensities of red against which the precipitates of Prussian blue, 10–20 μ in diameter, produced at the electrode tip show clearly.

The technique is simple and not very time-consuming to operate; it is possible to observe the site of recording 30 h after recording has been made. The technique shares the disadvantage with other marking techniques that only the last site of recording can be marked for identification unless it is possible to use several electrodes on each preparation.

I have pleasure in recording my thanks to Professor O. E. Lowenstein, F.R.S., for advice and encouragement, and to the Department of Scientific and Industrial Research for a maintenance grant during the period in which this work was done.

REFERENCES

Scheibel, M., and Scheibel, A., 1956. Stain Techn., **31**, 1.
Tasaki, I., Polley, H., and Orrego, F., 1954. J. Neurophysiol., **17**, 454.
Winsbury, G., 1956. Rev. Sci. Instr., **27**, 514.
Woldrings, S., and Dirken, M., 1951. J. exp. Biol., **28**, 218.