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TECHNICAL CONTRIBUTION

A DUAL MARKING TECHNIQUE FOR MICROELECTRODE TRACKS AND LOCALIZATION OF RECORDING SITES[1]

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The identification of fine microelectrode tracks and the localization of recording loci is a common requirement in neurophysiological studies Ideally, several criteria must be met for both recording and histological purposes (1) the techniques should allow the investigator freedom to mark several locations without damaging the recording characteristics of the electrode, (2) the physiological state of the preparation should not be drastically altered, (3) since multiple marks are frequently required, the marking process should be rapid, (4) electrode tracks should be identifiable under gross inspection during sectioning, and (5) tip localization marks should be small but easily identifiable Several techniques have been employed for both extra- and intracellular marking, ranging in complexity from simply running the electrode up and down in its track several times, to marking iontophoretically with various dyes (Thomas and Wilson 1965, Stretton and Kravitz 1968, Lee *et al.* 1969) While such techniques have been used satisfactorily in specific situations, no present method meets all of the above requirements The purpose of this paper is to report a method of marking in the brain that meets each of these criteria

A recent report from our laboratory described the use of horseradish peroxidase (HRP) for labeling single cells with extracellular recording microelectrodes (Lynch *et al* 1974) The present paper employs a similar procedure but with emphasis on creating a small extracellular HRP deposit to be used for electrode tip localization

METHODS

The techniques described in this paper consist of two separate parts (1) marking the electrode track, and (2) marking specific recording sites

Marking procedure

Marking electrode tracks is simply accomplished by coating the exterior of the microelectrode with a thin layer of concentrated fast green dye, dissolved in physiological saline The dye is carefully applied with a cotton tip applicator with one or two strokes directed from the electrode shank toward the tip, this can be easily accomplished without breaking or clogging the tip of the electrode When deep penetrations with the electrode are required the track can be seen more easily if the dye is allowed to dry on the electrode for 2 or 3 min before the presentation is initiated The electrode should be recoated prior to each plunge

During sectioning on a freezing microtome, electrode tracks marked by the method described above are easily identified There is some diffusion of the dye in fresh tissue, so it can be seen several hundred microns from the tract, enabling section(s) containing the track(s) to be anticipated Much of the diffused dye washes out during counterstaining so that only a very limited track profile remains green (Fig 1)

The extracellular recording sites are marked with HRP Glass micropipettes filled with 1% HRP in 2 *M* NaCl are first coated with fast green dye and then used in the conventional manner A discrete mark (25–50 μ) is easily made by passing 1–4 μA (anodal current) through the electrode for 4 sec. Several successive marks can be made during a recording session without altering the recording capabilities of the electrode

It is also possible to mark the electrode tracks with HRP (Fig 2) This can be accomplished by passing 2–4 μA anodal current while the recording electrode is slowly withdrawn from the tissue Since HRP is not visible in the tissue before it is developed, the simultaneous use of the fast green dye on the exterior of the electrode is extremely helpful in locating critical sections, thereby reducing the number of sections that must be developed

Tissue preparation

Following the completion of the recording session the animal is perfused with 10% formalin solution The perfused brain is then stored at 4°C Sections can then be developed for HRP immediately or stored for a week or more at 4°C Since fast green dye is water soluble, storing longer than 24 h reduces the amount of dye in the tissue Therefore, for best results, if only the electrode tracks are to be visualized the brain should be sectioned and stained within 2 or 3 days following the perfusion

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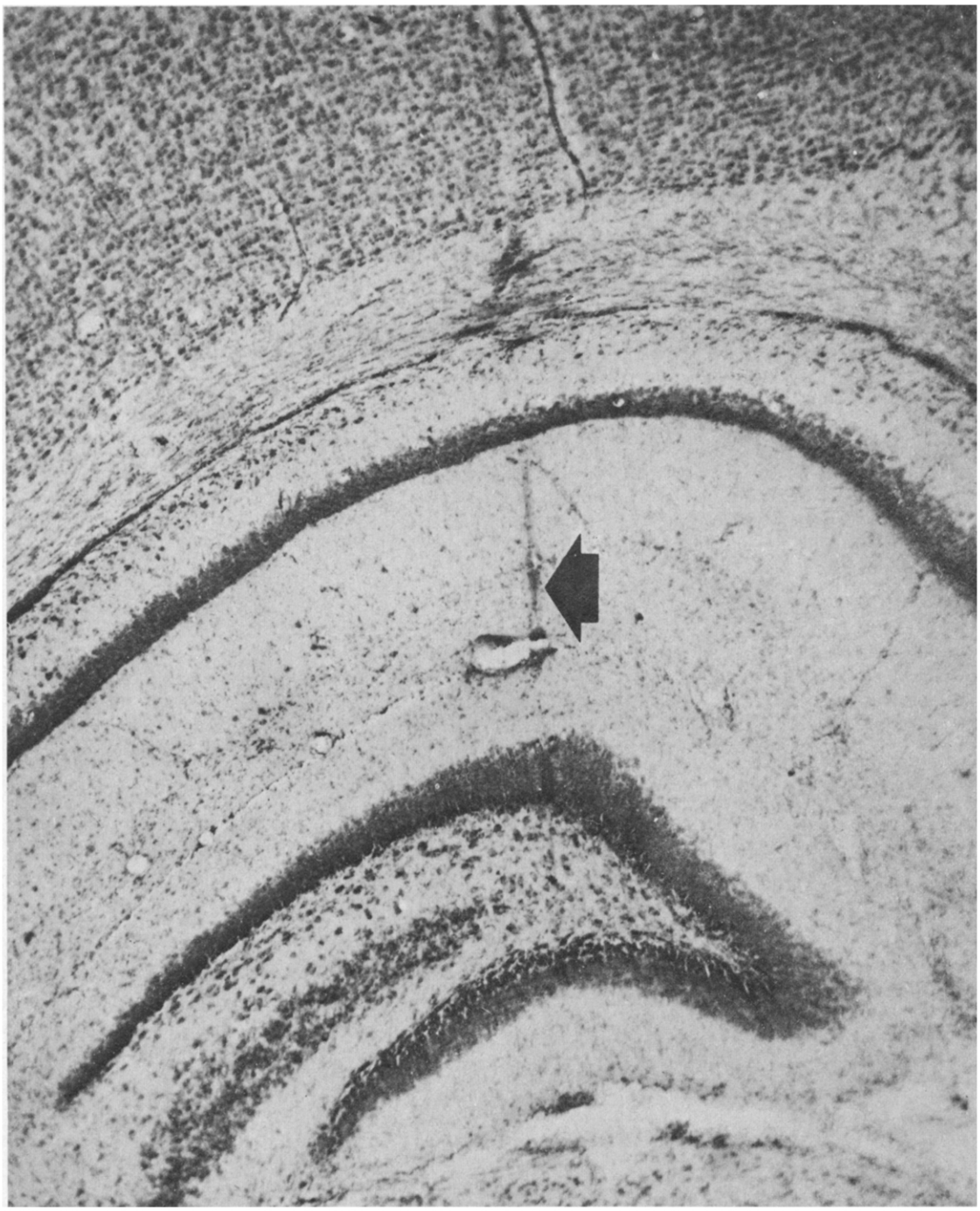

Fig 1 A low power photomicrograph illustrating microelectrode track (arrow) stained with fast green dye The section was counterstained with cresyl violet

Developing and counter-staining

To develop the HRP, sections stained with the green dye are washed in distilled water (5 min) and placed in the following incubation medium for 60 sec 0 03% hydrogen peroxide and 0 125% benzidene After incubation the sections are washed briefly in 35% ethanol (ETOH) and then placed into a dish containing 9% sodium nitroferricyanide for 15 min to stabilize the reaction product After stabiliza-

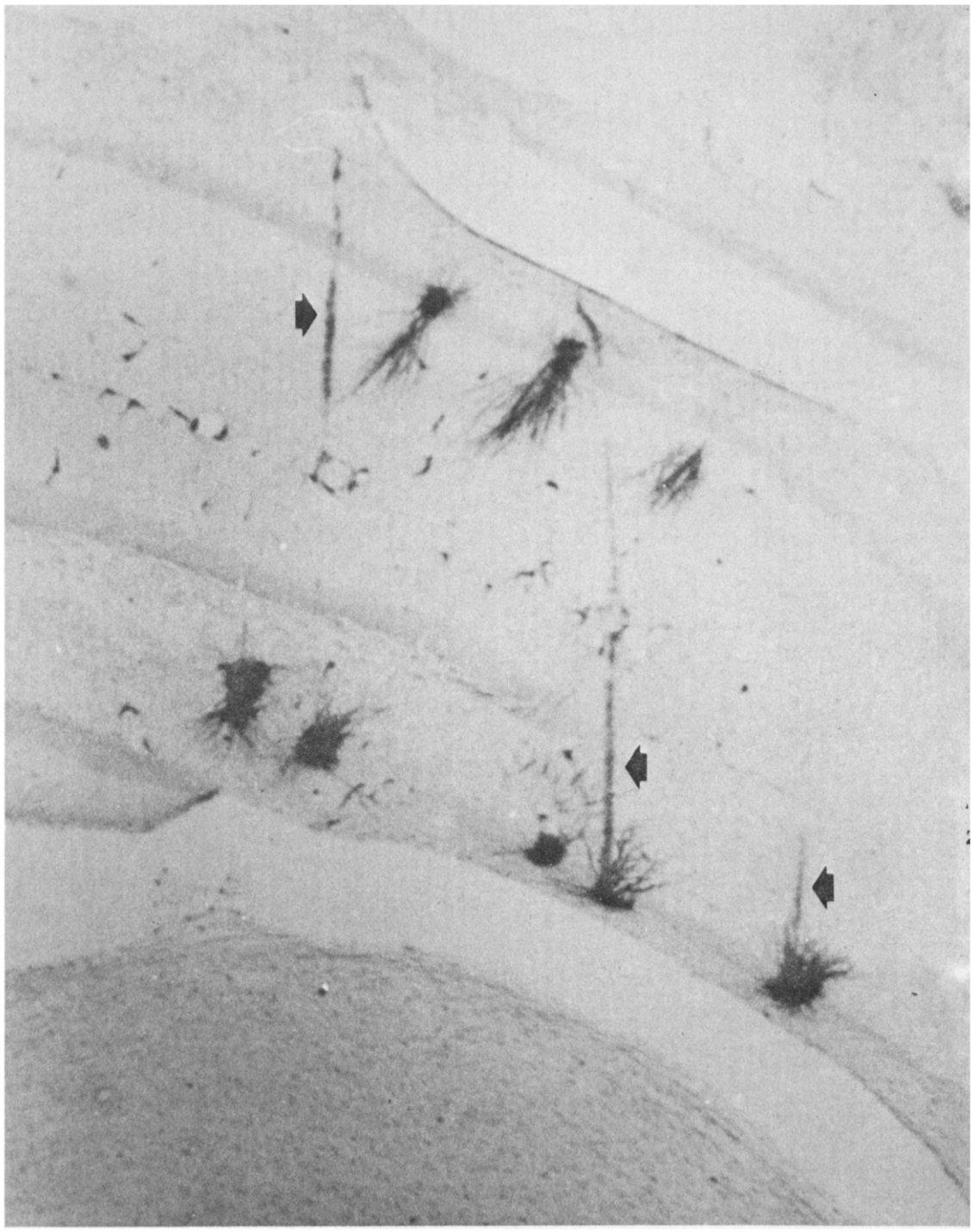

Fig 2 A photomicrograph illustrating multiple marks and microelectrode tracks (arrows) marked with horseradish peroxidase (HRP) The section was counterstained with saffranin All recording loci and electrode tracks were marked using the same recording electrode

tion, the sections are washed briefly in 25% ETOH, placed in distilled water and immediately mounted on slides After drying, the sections are counterstained with saffranin or cresyl violet and covered. This procedure results in sections in which the cell bodies are stained red, the electrode tracks are green (or blue), and the recording marks are dark blue.

DISCUSSION

These methods for marking in brain are extremely effective for pin-pointing recording sites The use of fast green alone can be employed in situations where the extent of penetration is the only verification required, or when fine metal electrodes are employed An incidental but helpful effect of coating the electrode with fast green dye is that the darker tip of the electrode can be visualized much more easily while it is being positioned prior to being lowered into the brain As described above the marking technique requires little time, this is in contrast to earlier methods (Thomas and Wilson 1965), which make marking even a moderate number of loci prohibitive The use of HRP for marking recording loci has an added advantage over other techniques Since the enzyme is taken up by neurons, marking often results in the labeling of several nearby cells, permitting the visualization of the cellular morphology at a particular recording locus

SUMMARY

This paper describes techniques for marking both microelectrode tracks and exact recording loci using a combination of fast green dye and horseradish peroxidase (HRP) The procedure involves coating the exterior of HRP filled microelectrodes with fast green dye in order to identify electrode tracks, and ejecting HRP from the electrode to mark recording loci Rapid, multiple marks can be made with this technique without harming the recording capabilities of the micropipette

RESUME

DOUBLE TECHNIQUE DE MARQUAGE POUR TRAJET DE MICROELECTRODES ET LOCALISATION DES POINTS D'ENREGISTREMENT

Ce papier décrit des techniques de marquage du trajet des microélectrodes et des points exacts d'enregistrement, à l'aide d'une combinaison de teinture verte rapide et de peroxidase de raifort (HRP) Le procédé comporte le revêtement de l'extérieur de micro-électrodes remplies de HRP par de la teinture verte rapide afin d'identifier les trajets d'électrodes, et l'éjection de HRP de l'électrode pour marquer le point d'enregistrement Des marquages multiples et rapides peuvent être effectués avec cette technique sans entraver les possibilités d'enregistrement de la micropipette

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