Letters to the Editor

AN IMPROVED METHOD FOR EXTRACELLULAR MARKING OF ELECTRODE TIP POSITIONS IN NERVOUS TISSUE

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Abstract. The Alcian blue method for extracellular marking of recording sites has been improved by filling the glass micropipettes with 3% Alcian blue 8GX or 8GS in 3M KCl buffered to pH 5.2 with 0.05M Potassium acetate. By filling the microelectrodes with a strong electrolyte (3M KCl) the same low resistances (5-25 MΩ) and recording properties as conventional micropipettes are preserved. Currents of 0.5-1 μA for 1-3 min passed through the different electrode connected as anode, resulted in coloured spots of 10-100 μm in diameter. There was a positive correlation between the amount of current passed, and the size of the spots. The localization of the electrode tips was successful in more than 80% of all cases. The dyemarks remained for at least 5 days in vivo and resisted many histological procedures.

Introduction. Several techniques for extracellular staining of recording sites by microelectrophoresis have been reported in the vertebrate brain (Godfraind, 1969; Hellon, 1971; Lee et al., 1969; Thomas and Wilson, 1965). None of these techniques were entirely satisfactory. The stain must have a good solubility and electrophoretic mobility in hydrophilic environments and in addition should bind strongly to the nervous tissue. Strong binding results in intensely coloured spots which will remain for several hours or days in vivo and endure all histological techniques to be employed. In addition, the micropipettes used for the marking technique should have the same recording properties as micropipettes routinely used in neurophysiological preparations.

Lee et al. (1969) reported a method with Alcian blue stain that met most of the above criteria. Unfortunately, micropipettes filled according to
this procedure have high resistances; much too high for good recordings and positive microelectrophoretic results. This letter describes a modification of the Alcian blue method that overcomes these disadvantages.

Methods. White house mice, *Mus musculus*, (Harnischfeger, 1977, 1978) and the bat *Rhinolophus ferrumequinum* (Schuller, 1979) were used to test the electrodes by extracellular recording of responses from single units to acoustical stimuli in the inferior colliculus.

The micropipettes were pulled from thoroughly cleaned borosilicate capillary glass with inserted filament (Hilgenberg Glas, D-3509 Malsfeld, article no. 1100104; outer diameter 1.5 mm, inner diameter 0.86 mm) and filled by boiling under vacuum.

The dye solution consisted of 3% Alcian blue 8GX (G. Gurr) or 3% Alcian blue 8GS (Carl Roth KG) in 3M KCl. The pH was maintained at 5.2 with 0.05M Potassium acetate and the solution was suction-filtered through paper filters and micropore membrane filters (smallest pore size: 0.2 μm, Sartorius Membranfilter GmbH). Micropipettes filled with this solution had exactly the same resistances as identical micropipettes filled with 3M KCl alone. Measured in Ringer's solution, the resistances ranged from 5 to 25 MΩ, depending on tip diameters and taper lengths (6-12 mm tested).

For microelectrophoresis, a current of 0.5-1 μA for at least 60 seconds had to be passed through the electrode; the electrode being the anode. An adjustable DC voltage source (50-300 V) in series with a 100 MΩ resistor and a microamperemeter was used to control the current required.

Frozen sections were cut at a thickness of 50 to 80 μm and stained with cresyl fast violet (Nissl-stain). The recording sites indicated by the Alcian blue spots could be determined under the microscope.

Results. The recording properties of the micropipettes containing dye did not differ from those of micropipettes filled with 3M KCl alone. The s/n-ratios of extracellular recorded action potentials were just the same as those recorded with conventional 3M KCl glass micropipettes. Comparable numbers of units per penetration were obtained and these units could be held as long as with conventional electrodes.

After electrophysiological data had been collected, the recording site were marked by passing positive current through the micropipette (see methods). Currents of 0.5-1 μA for 60-180 seconds resulted in discrete, intensely coloured spots of 10-100 μm in diameter. There was a positive
correlation between amount of current passed, and the size of the spots, e.g. for 30 \( \mu \text{Asec} \): 10 - 35 \( \mu \text{m} \); for 90 \( \mu \text{Asec} \): 25 - 50 \( \mu \text{m} \); for 120 \( \mu \text{Asec} \): 30 - 70 \( \mu \text{m} \); for more than 120 \( \mu \text{Asec} \): 60 \( \mu \text{m} \) and larger (Fig. 1).

Fig. 1: Photomicrograph of a sagittal section of 80 \( \mu \text{m} \) thickness through the colliculus inferior of the greater horseshoe bat, *Rhinolophus ferrum-equinum*. The picture shows five Alcian blue spots of 40 - 100 \( \mu \text{m} \) in diameter placed at the lower border of the nucleus and the adjacent region of the lateral lemniscus as indicated by arrows. Charges of 120 \( \mu \text{Asec} \) and more were passed through the electrodes during microelectrophoresis to produce large markings for easy recognition on the sections. When counterstained with cresyl fast violet the markings stand out deep blue against the violet neurons. Even on this black and white photograph, they can be distinguished solely by their optical density from artefacts produced by the electrode penetrations (central part of the picture). The five electrode tracks can be reconstructed by aligning the penetration injury at the surface of the colliculus (arrow) with the marking point. (The section was kindly provided by Drs. G.D. Pollak and G. Schuller.)

When charges of 30 \( \mu \text{Asec} \) or more were passed through the electrode, the probability of finding the marked point on the histological sections was better than 80%. This holds true only for the first dye ejection from the electrode. During the second or third attempt at microelectrophoresis, the electrode resistance rose steeply and prevented the passage of current.
through the microelectrode. To increase the probability for a second spot, the charge passed through the electrode should be minimized: the lowest amount of charge necessary for successful microelectrophoresis was determined to be about 30 pAsec (0.5 pA for 60 sec; Harnischfeger, 1977).

Since Alcian blue stain has a high binding capacity to the nervous tissue the dye spot can stay in vivo for at least five days (Fig. 2). This was the longest time tested.

![Photomicrograph of an Alcian blue marking of about 50 μm in diameter in the inferior colliculus of the house mouse, Mus musculus. The stain survived about 5 days (112 hours) in vivo.](image)

The dye markings were not affected by paraffinembedding and survived Nissl-staining, the peroxidase procedure of Graham and Karnovsky (1966) and the Bodian silver impregnation. The same result have also been reported for Golgi-Cox impregnations (Lee et al., 1969).

**Discussion.** Micropipettes filled with 3% Alcian blue 8GX in 0.5M Sodium acetate (pH 5.8) as described by Lee et al. (1969) always have resistances higher than 30 MΩ and our microelectrophoretic attempts failed. In our earlier investigation (Harnischfeger, 1977), micropipettes with resistances of 10 MΩ and less, as postulated by Lee et al. (1969), were only obtained by breaking back the tips to 2 μm or more. The application of 3M KCl as electrolyte buffered with 0.05M Potassium acetate at pH 5.2 improved the method greatly, i.e. the microelectrodes had resistances and recording properties indistinguishable from those filled with 3M KCl alone.
To obtain intensely coloured spots, the dye concentration should be about 3% but not less than 2%. The pH of the solution is not critical and can vary over a wide range. However, the dye precipitates in alkaline solutions (Scott et al., 1964).

A systematic study shows Alcian blue to have a high binding capacity to nervous tissue both in vivo and in vitro. Other dyes used for extracellular marking of recording sites, such as Pontamine blue (Godfraind, 1969; Hellon, 1971) and Fast green (Thomas and Wilson, 1965) do not exhibit this property (David S. Marsh, personal communication with regard to Pontamine blue and Lee et al., 1969 as to Fast green).

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REFERENCES


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