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## A simple and rapid method for improving recording characteristics using multibarrelled micropipettes

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A method for the simple and rapid fabrication of multibarrelled micropipettes with improved signal-to-noise characteristics is described. The process of silicone coating the exterior of the multibarrel assembly was found to improve recording characteristics greatly and to reduce recording noise during the passage of ionophoretic current. This simple process of fabrication and silicone coating is completed within 15–20 min and is technically undemanding.

### Introduction

Glass multibarrelled electrodes used for extracellular recording and ionophoresis suffer from having poor signal-to-noise recording characteristics when compared with single-barrelled electrodes, due to resistive and capacitive coupling between the glass barrels (Stone, 1985). The attenuation of bioelectric signals arises from the resistance of the microelectrode ( $R$ ) in combination with the capacitances of the microelectrode ( $C_e$ ) and that of the amplifier to which it is connected ( $C_a$ ) (Cornwall and Thomas, 1981). This capacitance ( $C_e + C_a$ ) and resistance ( $R$ ) together form a low-pass filter (Cornwall and Thomas, 1981) which attenuates high-frequency signals, limiting the signal bandwidth to a frequency ( $f$ ) where:

$$f = 1/2\pi RC$$

It follows therefore that a reduction in either  $R$  or  $C$  will increase the frequency bandwidth, so improving the signal-to-noise ratio. Attempts to construct improved multibarrelled microelectrodes have included using a metal-in-glass arrangement (Hellier et al., 1990) or glass-ensheathed carbon filament configuration (Armstrong-James and Millar, 1979). These methods reduce the resistance of the electrode quite substantially, and, therefore, increase the frequency bandwidth and enhance the signal-to-noise characteristics. Other attempts have included mounting a separate recording barrel onto the drug barrels (Crossman et al., 1974); however, these approaches have the disadvantage of being time consuming and technically demanding. Attempts to reduce the capacitance of recording electrodes are commonly applied to single electrode configurations. In patch-clamp recording, coating the outside of the glass recording pipette with silicone (e.g., Sylgard) is used to lower capacitance to ground and so reduce the background noise (Hamill et al., 1981). A similar protocol has been employed in single-electrode voltage-clamp recording in which a pitch-based sealing wax has

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been used successfully to reduce the electrode capacitance by up to sevenfold (Cornwall and Thomas, 1981). As yet little effort has been made to reduce capacitance in multibarrelled pipettes. In this report we describe a simple and rapid method for both the construction of multibarrelled micropipettes and the reduction of electrode capacitance by siliconising the outside of the multibarrelled micropipette assembly.

### Materials and methods

Five- or 7-barrelled microelectrodes (Fig. 1) were made from borosilicate glass capillary tubing with an inner glass filament ('Kwik-fil', 1.5 mm outer diameter  $\times$  0.86 mm inner diameter; Clark Electromedical Instruments, UK). The in-

dividual pieces of tubing were arranged into a cluster of 5 or 7 and held firmly at the top and bottom by a 4-jaw pin vice (Eclipse No. 124, UK) of a custom-made vertical electrode puller, with the centre tube being extended in the top chuck to form a stalk of  $\sim 1\text{--}2$  cm (Fig. 1, stage 1). The assembly passes through a heating coil, which enables the glass at the centre of the assembly to be melted gently while the lower pin vice is allowed to drop  $\sim 1.5$  cm by gravity until it reaches a mechanical stop. As the electrode assembly drops it is manually rotated through  $\sim 360^\circ$  (Fig. 1, stage 2), causing the glass tubes to fuse. The heating coil is switched off, the glass allowed to cool slightly and the electrode assembly is then recentered in the coil. The coil is reheated to a slightly lower temperature and the pipette is finally pulled, leaving a multibarrelled

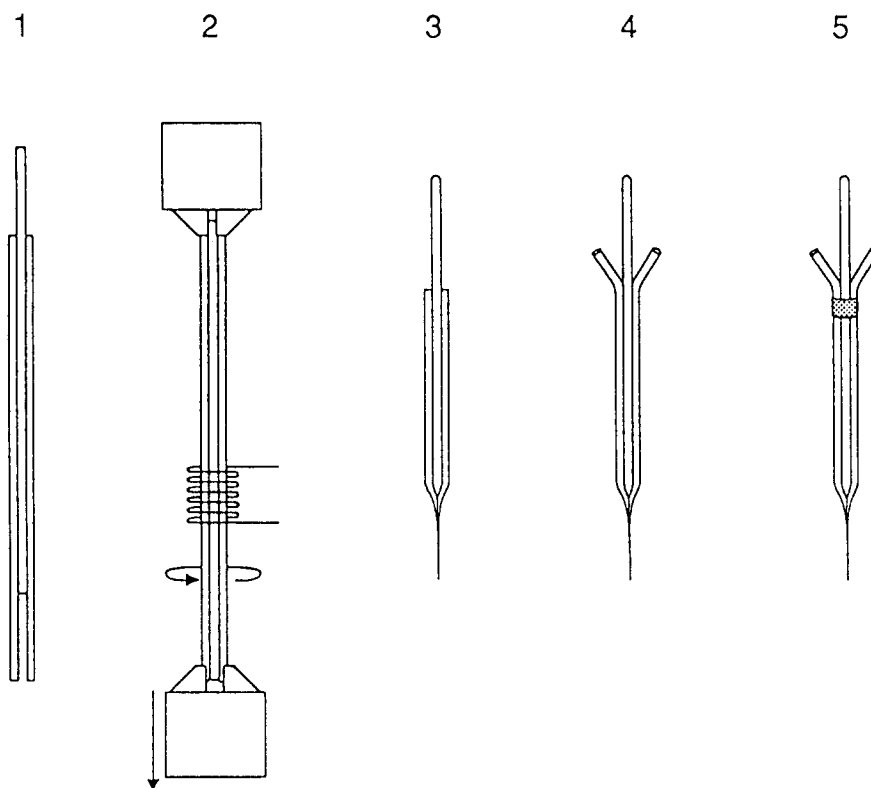


Fig. 1. Construction of a multibarrelled micropipette. The glass capillaries are arranged with the centre tube extended to form a stalk (stage 1). The assembly is then gently melted by a heating coil and allowed to drop  $\sim 11.5$  cm by gravity whilst being manually rotated through  $\sim 360^\circ$  (stage 2). After cooling, recentering and reheating the pipette is finally pulled, leaving an assembly with a protruding central capillary (stage 3). The side barrels are then flared (stage 4) and the assembly is strengthened with a collar of 'heat-shrink' tubing (stage 5).

assembly with a protruding central capillary (Fig. 1, stage 3) which is used for insertion into an electrode holder. The micropipette tip is then broken under microscopic observation to produce a tip of square profile with the desired diameter (4–8  $\mu\text{m}$ ) by placing the micropipette in a micro-manipulator and gently moving and bumping the tip against a flat surface. Finally the micropipette side barrels are flared by heating each barrel in a gas micro-flame and gently bending them away from the centre barrel (Fig. 1, stage 4). The flared barrels facilitate filling with solution, insertion of silver wires for the passage of ionophoretic current or the mounting of tubing for pressure ejection and prevent cross-contamination of the barrels upon filling. The barrel assembly is then strengthened with a collar of 'heat-shrink' tubing (4.8 mm diameter, RS components). The fabrication of such a multibarrelled pipette is readily completed within 5 min.

In earlier methods, fabrication of multibarrelled pipettes has required strengthening of the

assembly before pulling with a metal sleeve and/or epoxy adhesives (Crossman et al., 1974; Palmer et al., 1980) or has involved time-consuming and skilled techniques such as glassblowing (Salmoiraghi and Weight, 1967; Clark et al., 1978). However, in the present method, the need for these time consuming procedures are eliminated, since the 4 jaws of the pin vice form a circular arrangement to clamp the 5 or 7 tubes which enables the formation of a stable multibarrelled assembly for the pulling process. Rapid manufacture of multibarrelled micropipettes has also been successful using commercially available multibarrelled electrode pullers (i.e., the Narashige and Harvard models) modified with these inexpensive 4-jaw pin vices.

The centre barrel and one outer barrel were filled with potnamine sky blue (5% w/v in 0.5 M NaCl), the centre barrel being used for extracellular recording and the outer barrel for automatic current balancing, while the remaining barrels were filled with the drugs under study. The pre-

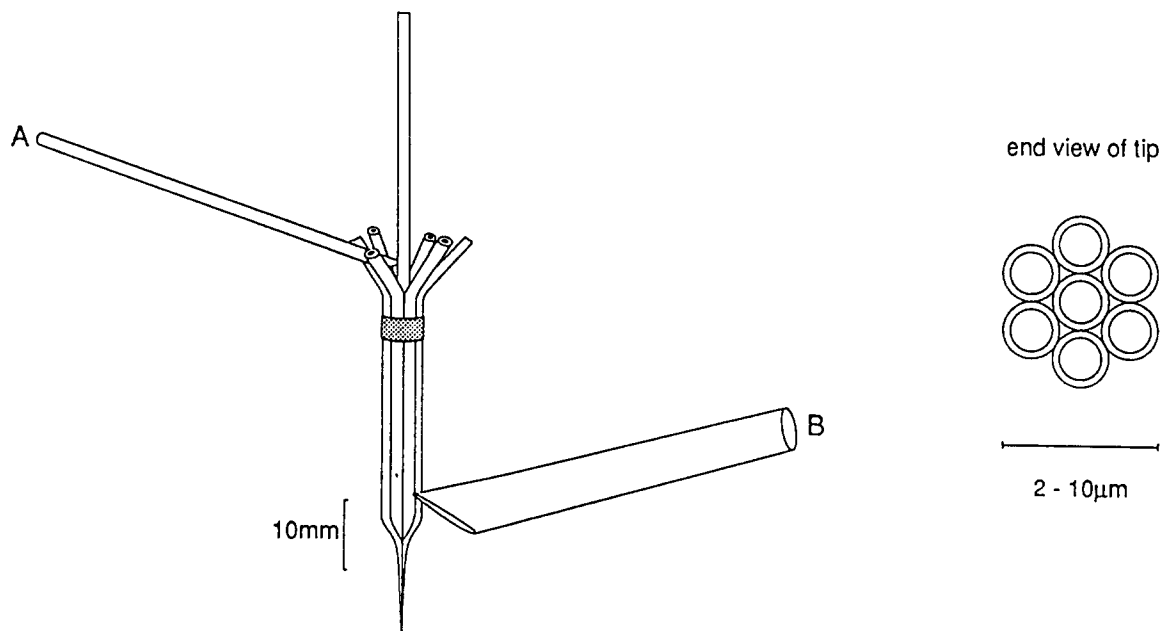


Fig. 2. Silicone coating procedure: silicone fluid is applied dropwise from a capillary (A) at the junction of the flared barrels. Any excess fluid is then wicked off at the shoulder of the assembly by a tissue (B), and the remaining solvent is allowed to evaporate at room temperature.

filled multibarrelled pipettes were then silicone coated by adding silicone (Sigmacote (Sigma) or Repelcote (BDH) were found to be equally effective) dropwise via a glass capillary at the junction of the flared barrels (Fig. 2). Any excess fluid is wicked off at the shoulder of the assembly with tissue paper (Fig. 2) and the solvent (Sigmacote: heptane; Repelcote: 1,1,1-trichloroethane) allowed to evaporate at room temperature over 10–15 min prior to use. Scanning electron microscopy and energy dispersal analysis were employed to determine the constituent elements at the electrode tip and revealed a silicone signal only, indicating that the silicone coating extends

to the pipette tip. The whole procedure of electrode fabrication, filling and silicone coating is readily completed within 15–20 min.

## Results

These multibarrelled micropipettes have been used to record from a range of CNS structures including cortex, hippocampus, thalamus and hypothalamus (Mason, 1986; Mason et al., 1991; Scott and Mason, 1991). It was found that compared with non-treated 7-barrelled micropipettes, the signal-to-noise ratio was greatly improved with

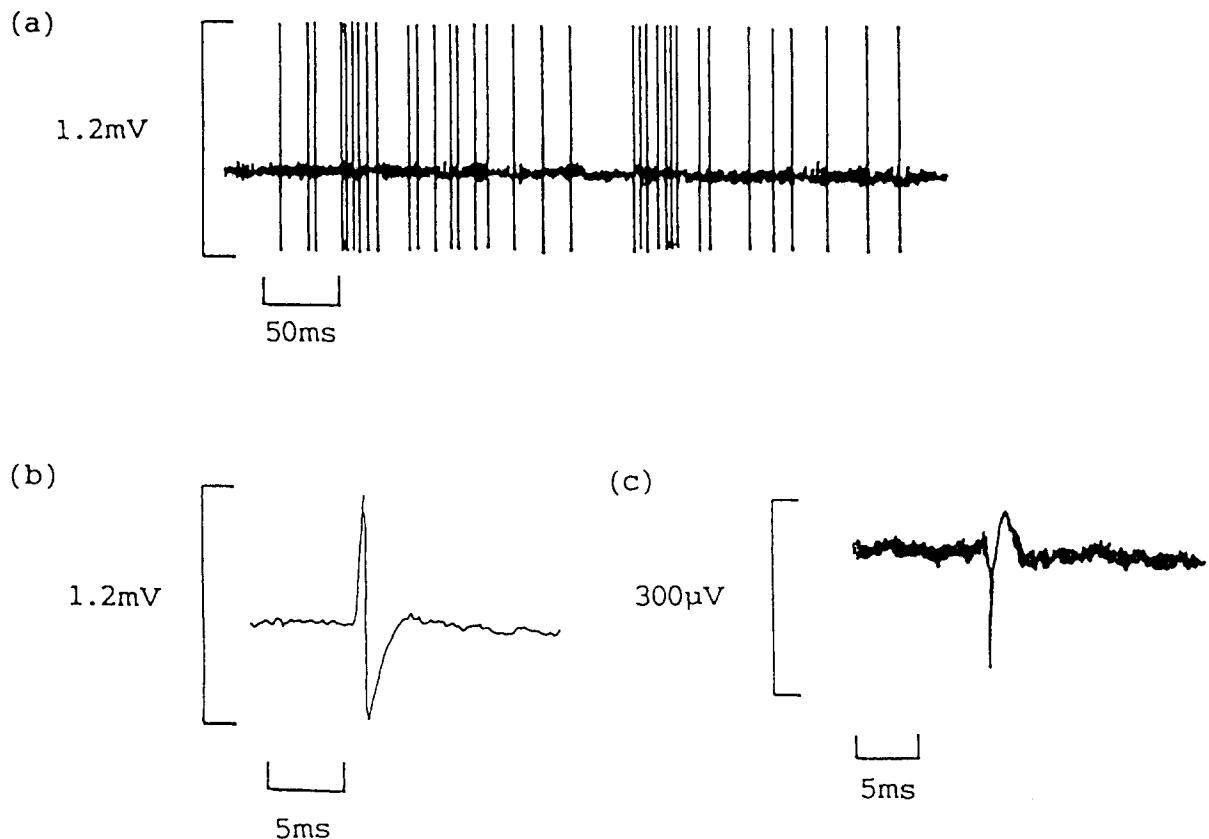


Fig. 3. a: Spike train recorded from a dorsolateral geniculate nucleus (dLGN) neurone in a urethane anaesthetised rat in vivo using silicone-coated 7-barrelled micropipettes. b: action potential from the same cell with an expanded time-base. c: action potential from a dLGN neurone recorded using a non-treated pipette. Extracellular neuronal activity was amplified ( $\times 20K$ ) and filtered with a Neurolog AC pre-amplifier (NL 104) and filters (NL 125: bandwidth 200 Hz–3.4 kHz) and displayed on a Tektronix 5000 series D13 dual-beam oscilloscope; photographic records of action potentials were made with a Shackman oscilloscope camera using Polaroid film.

spike amplitudes obtained up to 1.5 mV and a peak-to-peak noise level of 30–40  $\mu\text{V}$  (Fig. 3).

## Discussion

Silicone treatment did not alter the resistance of the recording barrels (2–5 M $\Omega$ ); therefore, it is possible that this treatment is in some way altering the capacitive component ( $C_e$ ) of the filter. The capacitance of a microelectrode is in reasonable agreement with that given by the relation for the capacitance of a cylinder (Cornwall and Thomas, 1981):

$$C_e = 2\pi e' e_0 / \ln(r_2/r_1)$$

where  $e_0$  is the permittivity of free space ( $8.8 \times 10^{-12}$  F/m),  $e'$  is the dielectric constant of the electrode glass ( $\sim 5$ ), and  $r_1$  and  $r_2$  are the inner and outer radii, respectively. Note that the value of the capacitance  $C$  depends only on the ratio of  $r_2/r_1$ , not their values. This would not appear to be the way in which our silicone treatment improves the recorded signal since there is no measurable change in the outer diameter of the pipette as viewed by scanning electron microscopy (data not shown). It is possible that this treatment may present resistive and/or capacitive coupling between the individual glass electrode barrels in the assembly. The treatment also prevents fluid 'creep' seen as wetting of the outside of the individual barrels of the assembly by physiological solutions; no saline, CSF or blood was observed to ascend the assembly by capillary action, unlike untreated pipettes. In addition, a major source of electrical noise is the capacitance between the electrode interior and this fluid meniscus that creeps up the electrode exterior. The meniscus is broken by this silicone-coating procedure, therefore contributing to the improvement in signal-to-noise characteristics. Whatever the mechanism of action, this silicone-coating procedure provides a simple and rapid way to improve signal-to-noise characteristics when using multibarrelled glass micropipettes, comparable to that found with carbon-fibre assemblies

when used in ionophoretic studies (Armstrong-James and Millar, 1979).

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