



Rapid coating of glass-capillary microelectrodes for single-electrode voltage-clamp

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Abstract

The single-electrode voltage-clamp technique requires sharp glass-capillary microelectrodes, whose electrical properties often limit the capabilities of the recording system. Here, we describe a rapid and simple way of coating fine microelectrodes with Dricote and Vaseline that improves their performance during voltage-clamp. The coating prevented clogging of the tips, improved the capacitance compensation of the electrodes, helped to seal the electrode tips into cell membranes and allowed visualization of the tips under saline solution. This new coating method led to greatly improved recordings and better characterization of the transduction and voltage-activated currents in an isolated preparation of spider mechanosensory neurons. © 1997 Elsevier Science B.V.

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1. Introduction

The concealed location and small size of many neurons precludes the use of double electrode voltage-clamp or whole-cell patch clamp for intracellular measurements (Sakmann and Neher, 1983), so that the only suitable approach is often via a single glass microelectrode. Modern single-electrode amplifiers allow accurate measurements of cell resistance and conductance (Suzuki et al., 1978; Weckström et al., 1991; Laurent, 1990, 1991; Juusola and Weckström, 1993; Laughlin and Weckström, 1993; Juusola, 1994), but their performance depends heavily on the electrical properties of the microelectrode. The switching, single-electrode voltage-clamp uses a time-shared control of voltage measurement and current injection to pro-

duce a membrane potential close to that of the voltage command (Wilson and Goldner, 1975; Finkel and Redman, 1984). Because of this rapid switching, capacitive charging of the microelectrode must be minimized and properly compensated. This places conflicting requirements on the microelectrode, whose impedance must be low enough to allow rapid switching, yet high enough for cell penetration and sealing to the cell membrane. One method of reducing the tip capacitance without raising the resistance is to coat the tip with insulating material. An additional problem may be changing tip resistance during an experiment, because of clogging or partial blockage of the electrode. This is a common phenomenon that is probably caused by organic materials in the tissue.

We have developed a simple, fast and effective method of coating microelectrodes that greatly improves tip behavior and voltage-clamping. The coating prevents clogging, increases the transmural tip insulation and enhances the membrane seal around the electrode.

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2. Materials and methods

2.1. Microelectrodes and the preparation

The microelectrodes were pulled with a Sutter P-2000 laser puller (Sutter Instrument Company, USA) from filament-containing borosilicate glass capillaries (Hilgenberg, Germany) with inner and outer diameters of 0.5 mm and 1.0 mm, respectively. The resistances of the microelectrodes, filled with 3 M potassium chloride solution, varied between 30 and 70 M Ω with the electrode in saline.

Dissection of the isolated spider slit-sense organ mechanoreceptor preparation and its adaptation for mechanical stimulation and single-electrode voltage-clamp have been described before (Seyfarth and French, 1994; Juusola and French, 1995a). Prior to neuronal penetrations, microelectrodes were lowered 0.5–1.0 mm into the saline by a manual micromanipulator (Leitz, Wetzlar, Germany). The time constants of the microelectrodes, τ_c , were measured from the exponential decay of the head-stage voltage pulses stored in the memory of a digital oscilloscope. In the saline, after dual capacitance compensation via the amplifier (SEC-1L, npi electronic, Germany) τ_c was 0.15–0.3 ms and in tissue typically 2–3 ms, resulting in a maximum cut-off frequency of ~ 60 KHz.

2.2. Coating procedures

Microelectrodes were coated with two different substances: Dricote (Dri; no. D140, Fisher Chemicals, Fisher Scientific, USA) and Vaseline (Vas; Vaseline Research Co., USA). The effects of coating were tested by using, (1) un-coated, (2) dri-coated, and (3) Dri-Vas-coated microelectrodes to record τ_c in the saline and changes in the transmembrane conductance of spider mechanoreceptor neurons during single-electrode voltage clamp.

Microelectrodes were first back-filled with the electrolyte (3 M KCl) before immersing their tips for a few seconds into Dri and allowing them to air-dry for 15 min with the tips slightly elevated. Dri contains $\sim 1\%$ silane dissolved in a mixture of $\sim 97\%$ methylene chloride, $\sim 1\%$ diacetone alcohol and $\sim 1\%$ *tert*-butanol solution, which evaporates at room temperature, leaving behind a polymerized layer of the Antispread (Järvilehto et al., 1986). Sufficient evaporation time was crucial, because wet Dri-electrodes usually caused decay of the receptor current within a few minutes, probably by releasing the toxic solvent methylene chloride. The anti-adhesive action of Dri-coating strongly reduced the clogging of the electrodes. This finding was significant for our work with this preparation, because some unknown substances from the tissue caused a sticky film on the surface of the bath, often irreversibly clogging

the tips of the electrodes. As the substances were released from the preparation, their effects accumulated, often being insignificant with the first electrode, but interfering with subsequent ones. Rinsing the bath was only partially effective in clearing the film. Microelectrodes with clogged tips displayed increased τ_c and insufficient capacitance compensation, making them unsuitable for voltage-clamping.

Dri-coated microelectrodes were fitted to the electrode holder and lowered into a separate Vas container adjacent to the micromanipulator so that about 3 mm of the tips became fully covered with Vas, before advancing them into the bath. The successive steps for coating the microelectrode tips are illustrated in Fig. 1A. Vas did not block the electrodes. This was confirmed by checking the resistance and compensated τ_c of the microelectrodes before and after the Vas dip (Fig. 1B).

2.3. Tip diameter

Scanning electron microscope, SEM, (Nanolab 2000, Bausch-Lomb, Canada) pictures were used to measure the tips of several microelectrodes with and without Dri. The tip diameter of the untreated electrodes was fairly constant (0.2–0.3 μm , $n = 6$). Dri-coating approximately doubled the wall thickness of the tip (Fig. 1C). Since capacitance is inversely proportional to wall thickness, Dri-coating would be expected to reduce the tip capacitance by a factor of ~ 2 . However, the advanced dual capacitance compensation of the amplifier was so effective that the coating had only a small effect on the observed τ_c (see also Richter et al., 1996).

SEM could not be used to observe Dri-Vas-coated electrodes because the high temperature and vacuum within an SEM would evaporate the Vas and damage the instrument. However, it seems certain that the additional layer of Vas increased the wall thickness even more and therefore reduced the actual tip capacitance further.

2.4. Recording procedures

Voltage-clamp experiments were performed after Tetrodotoxin (TTX) treatment, which blocked voltage-sensitive sodium channels and prevented action potentials. The TTX effect was complete in about 5–10 min, after which we first recorded the inward current that was produced by a mechanical displacement stimulus (i.e. the receptor current) and then the outward currents in the same mechanoreceptor neuron following voltage commands. Details of the stimulator and stimulation procedures with this preparation have been given elsewhere (Chubbuck, 1966; Juusola and French, 1995a,b).

Voltage-clamped current responses were recorded by a high impedance preamplifier (SEC-10L, npi elec-

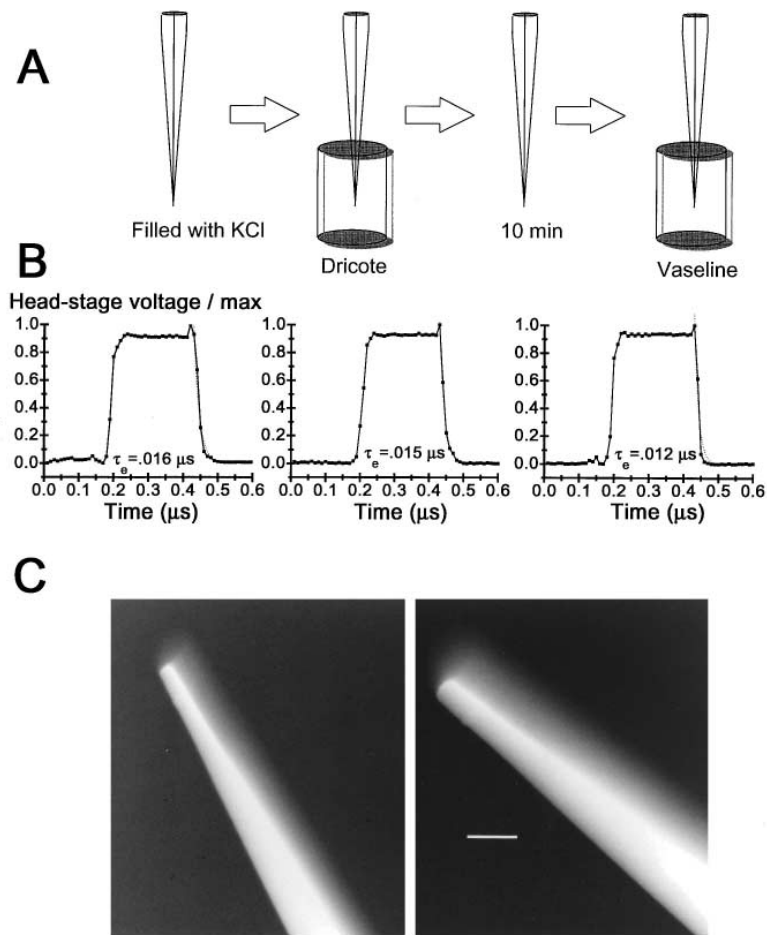


Fig. 1. Coating procedures and the electrical and physical properties of the microelectrodes. (A) Microelectrodes were filled with KCl. The tips were dipped into Dri and allowed to dry for 10 min, forming a white layer on the electrodes. Just before penetration, the tip of the electrode was dipped into Vas with the aid of a micromanipulator. (B) Normalized head-stage voltages from the same un-coated (left trace), Dri-coated (middle trace) and Dri-Vas-coated (right trace) microelectrode during ~ 10 kHz switching. The electrode resistance (from left to right: 37, 38 and 37 M Ω) and the compensated electrode time-constant, τ_e , measured in 1 mm depth of saline, did not change significantly because of the coating. (C) Dri-coating (right) approximately doubled the microelectrode tip diameter; left: the same microelectrode un-coated. Notice the same blemish on the left side of the tip in both pictures indicating that the tip was not damaged during the coating. Scale bar = 1 μm .

tronic, Germany; 1L headstage) and filtered with the corresponding voltage command at 2000 Hz (SEC-10L; low pass filter). The voltage command was injected via the recording microelectrode with a switching frequency of up to 20 kHz. In the mechanical stimulation experiments, the step stimulus and the resulting receptor current were also low-pass filtered at 2000 Hz. During each experiment the paired input and output signals were monitored on an oscilloscope, sampled at 2500 Hz, digitized with a 12-bit A/D converter (DT2821, Data Translation, USA) and stored in a computer (IBM-486 compatible 66 MHz). The sampling process was initiated simultaneously with the stimulus modulation produced by the computer and 300 to 500 ms records of both signals were obtained during each recording cycle. After a preset number of responses (usually 1–10), the average response was calculated. Data processing was done by custom written software

using the ASYST-language (ASYST 4.0; Keithley, USA) (Juusola, 1994; Juusola and French, 1995b). Because of the small τ_e and the effective dual capacitance compensation of the npI amplifier, the clamp error during SEVC experiments was minimal (see also Richter et al., 1996).

3. Results

Only neurons that demonstrated sufficient sealing, as judged by their resting membrane potential (≤ 65 mV), input resistance (> 40 M Ω) and action potential amplitudes (> 50 mV) 5 min after the electrode impalement, were selected for this study. When the electrodes had properly sealed to the cell membrane, the electrode coating had negligible effects on the cell input resistance (measured by injecting -0.1 nA current pulses; un-

coated: $68.1 \pm 35.4 \text{ M}\Omega$, $\mu \pm \sigma$, $n = 14$; Dri-Vas-coated: $60.4 \pm 21.3 \text{ M}\Omega$, $\mu \pm \sigma$, $n = 36$), but significant effects on the quality of the recorded membrane currents (see below). After TTX treatment, neurons were voltage-clamped to their resting potentials.

Coating the microelectrodes greatly improved the voltage-clamp measurements. Fig. 2 shows this effect, using receptor current recordings. When the electrodes were properly compensated, receptor current amplitude did not depend on switching frequency, indicating that tip capacitance was not limiting electrode performance. Dri-Vas-coated microelectrodes gave significantly larger responses under identical conditions to uncoated electrodes. However, the effects of Dri-coating alone on the amplitude of the receptor current were rather small. Although the size of the receptor current increased dramatically with Dri-Vas-coating, its dynamics were not affected. Fig. 3 compares typical responses of a Dri-coated microelectrode to those recorded with a Dri-Vas-coated one. Despite the obvious amplitude differences between the traces, the time-course and adaptation dynamics of the recorded receptor currents were very similar. In addition, the amplitude of the noise variance of the membrane potential recorded during voltage-clamp was approximately the same with coated and uncoated electrodes, while the noise in the receptor current traces was usually greater with Dri-Vas-coated microelectrodes. This suggests that the coating improved the dynamic resolution of the voltage-clamp, allowing the noise from receptor channels to be recorded. Similar observations were made with the outward currents evoked by depolarizing the membrane potential (Fig. 4). Outward currents were generally about 5 nA larger with Dri-Vas-coated microelectrodes than with uncoated microelectrodes.

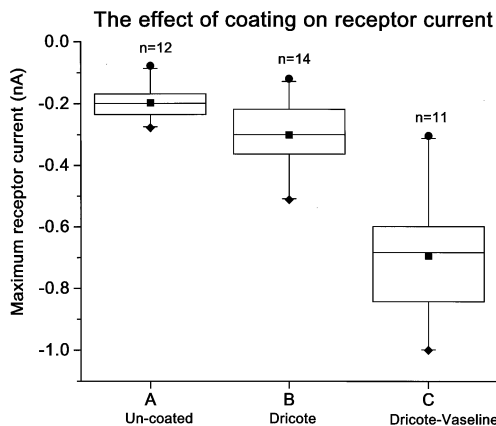


Fig. 2. Comparison of measurements made with un-coated, Dri-coated and Dri-Vas-coated microelectrodes. With un-coated microelectrodes, the average peak receptor currents were about 0.2 nA. The average values for Dri-coated and Dri-Vas-coated microelectrodes were about 0.3 nA and 0.7 nA, respectively. Mean, \blacksquare ; Max, \bullet ; Min, \blacklozenge .

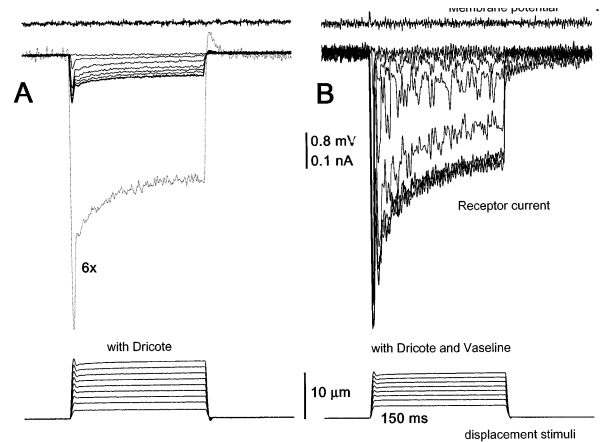


Fig. 3. Receptor current traces, 5-times averaged, recorded with Dri-coated and Dri-Vas-coated microelectrodes of similar input resistance. Dri-Vas-coating (B) produced larger receptor currents than Dri-coated microelectrodes (A). Note that the additional Vas-coating had only a negligible effect on the adaptation dynamics of the currents (compare the 6-times amplified trace to the largest trace of the Dri-Vas-coated microelectrode), but the noise in the recordings was greater with Dri-Vas-coated microelectrodes. The noise presumably arises from receptor channel activity. A similar maximum gain, with switching frequency of 20 kHz was used in both sets of recordings. Great care was taken to provide optimum capacitance compensation throughout the recordings.

4. Discussion

Dri-coating alone caused a reduction in electrode clogging, but Dri-Vas-coating of microelectrodes caused a more dramatic improvement in recorded receptor currents under voltage clamp in this preparation. There are several factors that could contribute to this effect:

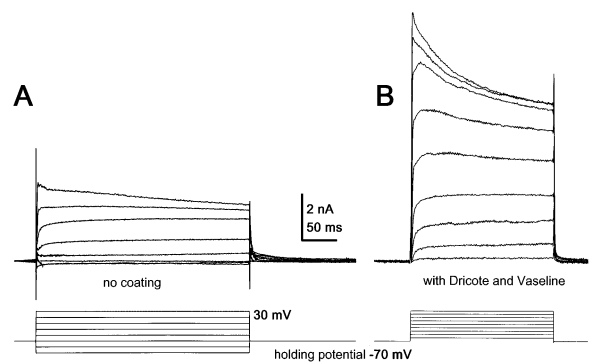


Fig. 4. Leak-subtracted, 10-times averaged outward currents recorded with un-coated (A) and Dri-Vas-coated microelectrodes (B). The maximum outward currents recorded with un-coated microelectrodes were typically $> 5 \text{ nA}$ smaller than with Dri-Vas-coated microelectrodes. Notably, the leakage currents recorded with un-coated and merely Dri-coated microelectrodes were larger than with Dri-Vas-coated microelectrodes.

4.1. Electrode capacitance

It is essential to keep the neurons surrounded by saline, so they are always covered by 0.5–1.0 mm of conducting solution. This increases the amount of the tip that contributes to the microelectrode capacitance. In time-sharing, single-electrode voltage-clamp amplifiers the input capacitance of the electrode is precharged with a neutralizing current, to prevent changes in potential from charging the capacitance. The transmembrane capacitance is distributed towards the tip of the microelectrode and is especially difficult to neutralize when rapid events require a fast switching frequency. Single-electrode amplifiers sample the potential at the electrode just prior to the onset of the current pulse. If the electrode transient has not decayed to zero at this time, the clamp becomes unstable for gains that are sufficiently large to provide meaningful results (see Finkel and Redman, 1983).

It is clear that the Dri-Vas-coating increased the effective microelectrode wall thickness at the tip and must have decreased the tip capacitance. However, the powerful dual capacitance compensation of the amplifier caused the apparent electrode time constant to remain relatively constant under all conditions. Thus, while the coating probably reduced tip capacitance significantly, it was difficult to observe any major changes in the switching clamp performance due to this factor.

4.2. Electrode clogging

During dissection and recording, a substance is released from the spider tissue that easily clogs the tips of the electrodes. This increases the input impedance and τ_c of the electrode, degrading the recording by reducing the available voltage-clamp gain and the switching frequency. With good capacity compensation, minor electrode clogging should not lead to lower currents because the switching clamp reduces the apparent access resistance to zero (Jarolimek and Misgeld, 1993). Dri-coating or Dri-Vas-coating caused a major reduction in electrode tip clogging and therefore improved current recordings. The reasons for this are not immediately obvious, but could be related to the polar properties of the substances that normally clog the electrode. The Dri- or Dri-Vas-coating could either prevent polar substances from reaching the mouth of the electrode, or could absorb hydrophobic substances before they reach the mouth.

4.3. Tip damage

A tough glial layer wraps the neurons and often breaks the electrode tip. Depending on the magnitude of this tip damage, the microelectrode may still be

suitable for penetrating the membrane, but often becomes too large for successful recordings. Although Dri-Vas-coating significantly increased the tip diameter, there was no increase in the occurrence of tip breaking and no significant change in input resistance with coated microelectrodes. The hydrophobic nature of the coating may therefore improve the ability of the electrode to pass through the glial membranes.

4.4. Visualization

Although the neurons can be seen with good illumination, it is difficult to determine the exact location of the tip before touching the neurons. This increases the risk of tip damage. Dri-Vas-coating improved this situation considerably, making it much easier to estimate the location of electrode tips.

4.5. Space clamp

Although the coating may have reduced tip capacitance by increasing wall thickness and reduced tip resistance by preventing clogging, it is still difficult to explain the dramatic improvement in current amplitude. The changes in the recordings were similar to what would be expected from an improvement in the space-clamp of the neurons. This would allow more distant regions of the cell to contribute to the recorded current. Since spider slit-sense organ neurons have up to 150 μ m long dendrites and the site of transduction is presumed to be at the dendritic tips, an improved dendritic space-clamp would significantly increase the recorded receptor current and allow improved temporal resolution of the mechanotransduction events. One way in which the space-clamp could be improved would be for the Vas to spread over the somatic region of the neuron, reducing its conductance and capacitance. However, an argument against this possibility is our failure to observe any changes in input resistance or in the dynamic properties of the outward current.

4.6. Previous use of Vas

Vas is commonly used when recording from sensory neurons in insect compound eyes and thoracic ganglia. However, in these cases the microelectrode coating is unintentional, because the Vas is used to prevent tissue dehydration and incidentally coats the microelectrodes as they enter the tissue. It is interesting to note that, despite initial doubts about the clamp quality of many insect preparations, their sensory neurons have usually been quite amenable to voltage-clamp via the single-electrode technique in preparations where Vas is used to prevent dehydration (Hardie and Weckström, 1990; Weckström et al., 1991; Laurent, 1990, 1991; Juusola and Weckström, 1993; Laughlin and Weckström, 1993).

In fact, it was the unexpected initial difference in the quality of the outward current recordings between insect visual cells (see Juusola and Weckström, 1993) and spider mechanoreceptors that first made us suspect that Vas-coating might affect the quality of recording. As the data presented here show, simple coating of microelectrode tips with Dri-Vas leads to dramatic improvements in the quality of microelectrode recording.

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