

Chapter 1

Using microelectrodes

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1. Introduction

Microelectrodes are the basis of the techniques discussed in this book. They are used for: (1) potential recording; (2) current injection; (3) introduction into the cell of ion-selective resins for measuring potential or determining the free concentration of cytosolic constituents.

(1) and (2) are the procedures underlying conventional microelectrode recording, voltage-clamping and patch-clamping. This chapter will be restricted to considering the fundamentals of reliable and accurate measurement of membrane potentials and the experimental manipulation of membrane potential by injection of current. The specialized techniques involved in voltage-clamping and patch-clamping will be treated separately in subsequent chapters, as will the special requirements involved in the use of ion-sensitive electrodes or ionophoresis of drugs.

2. Making microelectrodes

One definition of a microelectrode (ME) might be: 'an electrode constructed with a tip having the dimensions of the order of a micrometre (1 μm)'. Usually this means a glass micropipette of the type pioneered by Ling & Gerard (1949), which is filled with an electrolyte solution to act as a conductor of electricity.

Glass MEs are made by heating a capillary until molten, when it is stretched; while the glass is still plastic but cooling down, the tip draws out, breaks and separates.

(i) By hand: This is not recommended because of lack of reproducibility, although great artists can heat a capillary in a bunsen and pull out a fine tip!

(ii) Vertical puller: This usually has a nichrome filament and a 2 stage pull - the first by gravity, the second electromagnetic. This is fine for MEs up to about 30 to 40 $\text{M}\Omega$. In our experience these pullers are less effective for making fine-tipped microelectrodes for use on small cells.

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(iii) Horizontal puller 1 (Livingstone type): These pullers are gear driven and have a platinum foil heating element. They are good for fine tips (ME resistance can be 30-300 M Ω) but this type of puller produces rather long wispy shanks.

(iv) Horizontal puller 2 (Brown-Flaming, Ensor, Industrial Science): These pullers usually have a platinum or nichrome heating element with a range of preheat times and a 1 or 2 stage pull. The Brown-Flaming has a gas jet which cools the heater rapidly. Good reproducibility can be achieved with these pullers, but setting them up correctly is time consuming. They are generally good for fine MEs with resistances up to 300-500 M Ω and short shanks.

3. Filling microelectrodes

Glass microelectrodes are usually filled with a salt solution. The composition of the solution can be determined by the individual experimenter and depends on the experimental protocol. Nowadays, the preferred method of filling is to pull electrodes from capillary that has a glass-fibre fused into the lumen. When the ME is pulled the lumen shape is preserved up to the tip. Using fibre-containing capillary, MEs can be backed-filled with small amounts of solution. The solution tracks down the channels formed either side of the fibre right down to the tip. Bubbles *do* form but don't occlude the lumen completely. The exception is when a bubble forms directly in front of an Ag/AgCl sintered pellet in a perspex ME holder. This is easy to remedy.

One should realize, however, that other forms of ME than this exist and that even with what might be considered to be a micropipette there are alternatives to an electrolyte solution as a conductor. Thus, micropipettes have been filled with molten Wood's metal which solidifies to give a continuous metal conductor (in our experience, simultaneously cracking the insulating glass envelope!) (Gesteland *et al.* 1965), or have been drawn over single carbon (graphite) fibres around 7 μm in diameter (Armstrong-James & Millar, 1979) to form a ME with a carbon conductor. These manufacturing techniques produce electrodes with impedances of 200 k Ω to 2 M Ω . Similar values are obtained with glass or varnish-coated tungsten MEs which have been electrolytically etched to a fine tip (Merrill & Ainsworth, 1972); it is possible to electroplate the tips with other metals for positional marking (e.g. iron, which can be visualized by the Prussian Blue reaction) or for lower noise and reduced polarization (e.g. Pt and Pt black). All these MEs which use non-electrolyte filling as the electrical conductor have high DC resistances, however, and are employed in *extracellular* recording and stimulation; in this recording mode they are used for registering the occurrence rather than the accurate wave form of signals (for example, neuronal discharges). An exception to the latter statement is the use of carbon fibre electrodes to measure redox potentials of oxidizable compounds in biological tissues. Metal electrodes behave electrically in moist preparations as if they are a small resistance in series with a larger resistance and parallel capacitance: they are not suitable for measuring standing DC potentials. They do, however, have low noise at the frequencies where most of the power from action potential signals is

concentrated. Consequently, a good signal-to-noise ratio is obtained when they are used for extracellular recordings, better than that of electrolyte-filled micropipettes.

Electrolyte-filled (usually with NaCl) micropipettes are also used for extracellular recording of neuronal discharges since they can also faithfully reproduce the potential wave-form down to DC levels. Frequently, these MEs are used to measure potentials set up by synaptic current flow across the resistance of the extracellular space. By considering the potential gradient and its spatial derivative within a tissue, the location of sinks and sources of current can be used to pinpoint synaptic regions within the tissue. This technique is known as current density analysis and the reader is referred to several articles for a complete treatment of the subject (Rall & Shepherd, 1968; Hubbard *et al.* 1969; Llinas & Nicholson, 1974; Nicholson & Freeman, 1975; Nicholson & Llinas, 1975).

4. Connection of microelectrodes to recording circuit

The preparation and the electrode are both wet; electronic circuitry is dry and has metallic conductors. Plain metal/liquid interfaces display junction potentials and can produce gas (hydrogen and oxygen) if current is passed through them. The latter is particularly annoying since the presence of gas bubbles on the electrode simulates the insertion of a capacitance in the circuit at the liquid/ metal junction, thereby limiting DC recording. (Hence the reason that metal MEs are not used for intracellular recording - see above). Connections are therefore made to the recording circuit via non-polarizable reversible electrodes. Silver/silver chloride (Ag/AgCl) electrodes exchange electrons for Cl^- ions in solution. They are usually employed in the form of silver wire coated with silver chloride or a sintered pellet of metallic silver and powdered AgCl pressed around a silver connecting wire. The pellets have the advantages of large current carrying capacity and stability to light. Stability is obviously of prime importance in measuring membrane potentials as is the property of reversibility, i.e. the property whereby the passage of current in either direction through the electrode does not alter the potential difference between the metal and the solution. The Ag/AgCl reference electrode is reversible and of constant potential because AgCl is sparingly soluble and therefore the solution is saturated with respect to AgCl; the concentration of Ag^+ ions in solution is inversely proportional to the $[\text{Cl}^-]$ (from the definition of solubility product) and as a result the potential E_{Ag} of metal relative to the solution is given by $E_{\text{Ag}} = \text{Const} - \text{RT/F} \ln[\text{Cl}^-]$ (from the Nernst expression for the electrode potential of a metal). Differences in $[\text{Cl}^-]$ in the solutions composing these 'half-cells' lead to a standing potential which should not vary unless $[\text{Cl}^-]$ does at either electrode. If, in the course of an experiment, $[\text{Cl}^-]$ does vary (for instance, as a result of changing the bathing medium in an in vitro preparation) then the AgCl reference electrode should be interfaced to the preparation by means of a salt bridge which will maintain a constant $[\text{Cl}^-]$, avoids damage to the AgCl electrode and toxic effects of Ag in the bath. The salt bridge comprises 1-2% agar in 0.15 M NaCl, or with continuous bath perfusion, concentrated KCl may be used to minimise junction potentials (see below). Normally the ME tip itself forms the other salt bridge, with a AgCl wire inserted in the ME barrel, but to

equalize the reference electrode potentials exactly an additional salt bridge may be used at the back of the ME, with a AgCl pellet in the ME holder.

AgCl pellet electrodes can be purchased cheaply and are available as discs or pellets, some small enough to insert in the back of wide bore ME glass. There are 3 widely used procedures for chloriding Ag wires; for each the wire must be clean. (1) Electrolytic coating is done by connecting 2 wires immersed in 0.1 M HCl to the poles of a 1.5 V battery and passing current for 20 minutes or so, reversing the polarity at regular intervals, resulting in a uniform but fragile grey coat. (2) Wires may be dipped in molten AgCl (requiring an intense gas/air or gas/oxygen torch and crucible) to produce a tough coating. (3) Ag wires kept in hypochlorite-based bleach become coated with AgCl, and can be changed frequently if necessary.

5. Junction potentials

There is a potential difference set up at the interface between two salt solutions of different ionic composition or concentration by differing diffusional flux of anions and cations across the boundary: this is a liquid junction or diffusion potential. It is described, for a single solute, by equations in the form

$$V = \frac{u - v}{u + v} \frac{RT}{F} \ln \frac{c_1}{c_2}$$

where u is the mobility of the cations in solution and v is the mobility of the anions and c_1 and c_2 are the solute concentrations on opposite sides of the interface. R is the gas constant, T , the temperature and F is Faraday's Constant. Equations for the junction potential in more complex cases are given by Barry & Lynch (1991). Junction potentials develop:

(1) as a result of different anionic and cationic mobilities and

(2) different solute concentrations. They develop between the bath solution and reference salt bridge if these differ, and a modified form of junction potential, termed a tip potential (see below), exists across the ME tip. They cannot be eliminated but the correct electrode configuration ought to aim to stabilize them with respect to experimental solution changes or to reduce them by trying to equalize u and v . A reference salt bridge of 150 mM NaCl and agar is suitable if the composition of the bath is constant.

Experiments in which changes of ionic composition are imposed are often made to determine reversal potentials, and precision is important if permeability ratios are to be derived. The junction potential change at the reference boundary can be minimised by a continuously renewed 2-3 M KCl junction. K^+ and Cl^- have similar mobilities in aqueous solution. Moreover, with high concentrations of KCl, diffusion of KCl from the salt bridge predominates and potential changes due to alterations in salt concentration in the bath are small. A small continuous outflow of KCl solution, generated by 1-2 cm hydrostatic pressure through a 2-5 μm tip, prevents dilution within the bridge by the bath solution and the resulting generation of a concentration

gradient and junction potential within the salt bridge and reference electrode itself. However, leakage of KCl into the solution bathing the preparation can lead to unwanted changes of ionic composition, so downstream siting and perfusion of the bath are essential. If this cannot be done, the electrode arrangement can be used to measure junction potentials at the tip of a NaCl bridge when the bath solution is changed in the absence of the preparation, and the results used to correct the membrane potentials recorded when the NaCl bridge is used as reference.

A second problem arises when kinetic measurements of membrane potential response to ionic changes are made, or when the bath solution is changed locally (i.e. not at the reference), for instance with a puffer pipette or similar device. In both cases a junction potential will exist within the bath, even if only transiently, between the recording site and the reference, and will contribute to the potential recorded. As an example, when recording the timecourse of Cl⁻-evoked potential changes by fast perfusion in skeletal muscle fibres, Hodgkin and Horowicz (1960) used differential recording with respect to a blunt ME placed adjacent to the recording site to avoid transient Cl⁻ junction potentials along the bath.

6. Tip potentials

The surface chemistry of the glass is complex: at the tip of a ME it is believed that a restriction of anionic mobility is established. As a result of this, the liquid junction potential is altered by properties of the electrode tip. This is the tip potential (TP). The TP is abolished by breakage of the tip. It can be measured by measuring the potential change when the tip is broken, or by registering the potential change when an electrode of similar manufacture and filling solution, but with its tip broken is added in parallel between the input of the measuring device and the solution bathing the ME tip. A list of TP properties is given in Purves (1981): TPs are (1) of negative sign, up to -70mV with electrodes filled with 3 M KCl and tested in physiological saline; (2) abolished when inside and outside solutions are the same; (3) larger in higher resistance electrodes; (4) may vary widely in magnitude between otherwise similar electrodes; (5) can often be reduced by filling MEs with acidic solutions; (6) can be reduced and even reversed in sign by addition of small concentrations of polyvalent ions (e.g. thorium, Th⁴⁺) and high concentrations, 10-100 mM [Ca²⁺], to the bathing solution.

The TP is the main source of uncertainty in measuring membrane potential because it changes depending on the immediate ionic environment into which the tip is placed. Adrian (1956) studied some properties of TPs of KCl-filled MEs and the change in TP when the tip was in 100 mM NaCl or 100 mM KCl (to simulate cell impalement). The TP is smaller on going from extracellular to intracellular solution, the difference becoming larger the larger the initial potential. For a TP of -5 mV, the error is about 2 mV. Roughly speaking, TP is proportional to ME resistance, and inversely proportional to salt concentration of the external solution. The change in TP in going from one solution to another is proportional to the size of the TP. Thus, if possible, MEs with the smallest resistance, and therefore small TP, should be selected. Adrian used MEs filled by the method of boiling; subsequently it has been

shown (Okada & Inouye, 1975) that the method of ME filling and also the time after filling influences TPs. Smaller TPs are observed with the fibre-filling method (e.g. MEs made from filament glass) and with immediate usage.

Clearly, the problem of TP is complex and unpredictable and possibly for this reason is largely ignored in the majority of research reports. Generally speaking, TP is negative and the effect on impalement is to estimate a more positive value of the resting potential as the TP becomes more positive inside the cell. Measurement of the TP requires use of a KCl bridge between AgCl electrodes at both the reference and ME connections. The potential is initially zeroed with KCl connections into the bath solution and the TP recorded as the additional potential when the ME is introduced. A system with differential recording

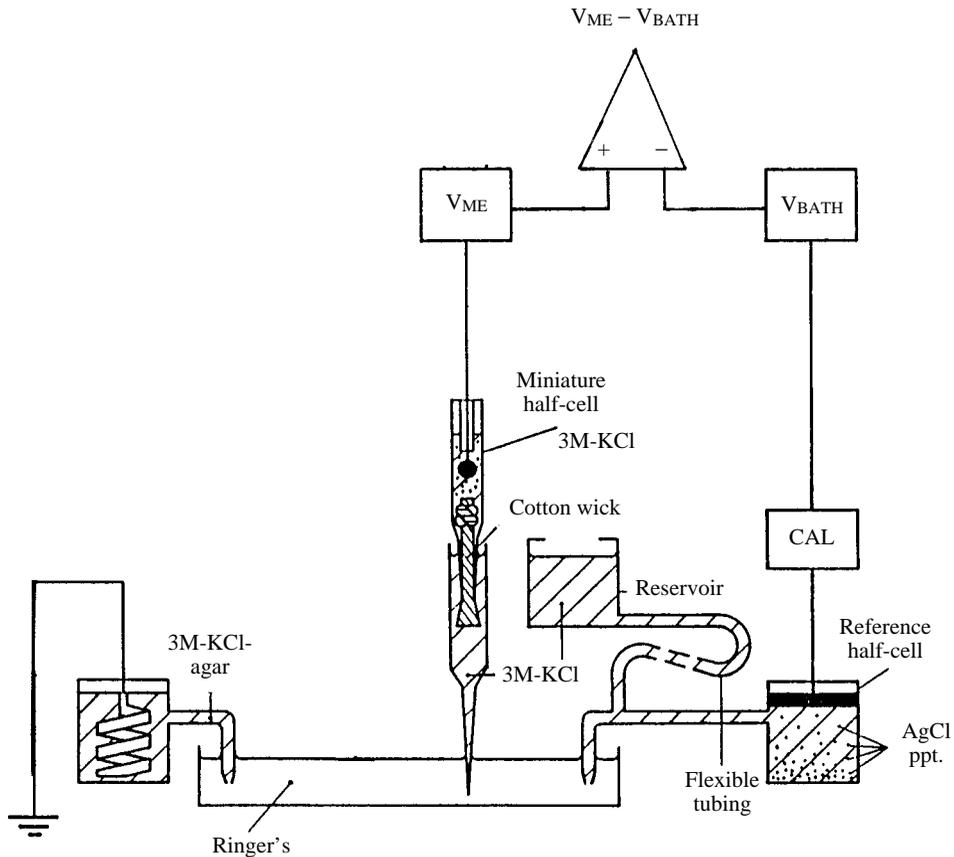


Fig. 1. An arrangement of electrodes permitting measurement of TP or junction potentials in a perfusion bath. The potentials of the reference half electrode and miniature KCl half cell are zeroed with respect to ground with the cotton wick in the bath. Placing the wick into the back of the ME introduces an additional potential due to the TP. During an experiment with changes in the bath composition the flowing KCl reference maintains a constant bath potential. Junction potentials with respect to normal Ringer can be measured by substituting a blunt pipette containing Ringer for ME and changing the bath solution. The calibrator CAL can be used to offset the standing membrane potential to look at small changes e.g. synaptic potentials at high gain without high pass filtering (D. C. Gadsby and D.C.O., unpublished data).

between ME and reference and KCl junctions to permit measurement of TP (or junction potentials of salt bridges introduced in place of the ME) is shown below.

Previous paragraphs have been included as an introduction to the wider range of ME usage; the recording techniques to be described are not dependent on a need to know the absolute value of potential. Even in the case of extracellular field potential analysis it is the difference of potential within the preparation that is of interest and any offsets or error potentials in the recording circuitry are of no consequence provided that they are fixed. It is, however, important to realize that using electrodes in aqueous solution to measure potentials is by no means as theoretically straightforward as putting a voltmeter across the terminals of an electronic component. The theoretical basis of potential measurement in aqueous solution is discussed lucidly by Finkelstein & Mauro (1974).

The rest of this chapter concentrates on using microelectrodes to measure cell membrane potentials.

7. Measuring membrane potentials using microelectrodes

The electronic amplifier used to record potentials via MEs should have the following characteristics:

(i) Input resistance should be at least 100-1000 times the ME resistance in order to measure the full signal at the ME tip and draw negligible current from the signal source.

(ii) Low leakage current. The current flowing into or out of the input terminal of the amplifier should be less than that which would cause a 1 mV drop across the ME or the cell input resistance.

(iii) Response time should be adequate: with a good response time there are ways of overcoming the low-pass filtering properties of a voltage recording set-up.

Capacitance compensation

The low pass filtering properties of the microelectrode and its amplifier are due to inevitable capacitances between the microelectrode and ground (Fig. 1). C_t , the transmural capacitance can be minimized by (i) thick pipette walls (glass pipettes can be made thicker by coating with a layer of Sylgard (Corning) and (ii) a low solution level in the bath to reduce the effective transmural area. C_s , the stray capacitance from the microelectrode and amplifier input to ground (that is, the microscope, bath, stand, etc.) can be reduced by minimizing the length of the ME and connecting wire and by driving the shield of the connecting wire with a low impedance signal from the output of the ME amplifier (Fig. 2). C_a , the input capacitance of the amplifier should be negligible if the amplifier is chosen to have a good response time as suggested. C_{tot} is the total summed capacitance (see Fig. 1 and Chapter 16).

Once C_{tot} is minimized it can be compensated for by using a feedback circuit often described as 'negative' capacitance (Fig. 3). This positive feedback circuit provides the current lost through C_{tot} , preventing a potential drop across the electrode

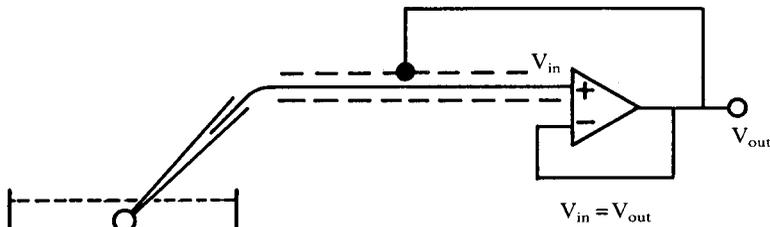


Fig. 2. A low-impedance driven shield to reduce C , the stray capacitance to ground.

resistance. Good compensation clearly depends on the rapidity with which the feedback circuit can supply current. The fully compensated rise time is proportional to the geometric mean of the rise time of the recording amplifier and the rise time of the uncompensated circuit. This suggests that the best strategy is (i) to minimize stray capacitance and (ii) use a head-stage amplifier with a fast rise time. Over-compensation of input capacitance results in damped oscillations at the leading edge of potential steps and finally in continuous oscillation.

8. Manipulating cell membrane potentials

Experimental protocols often require the manipulation of membrane potential (for example, to test passive membrane properties such as input resistance by passing current into the cell). With two MEs in the same cell, one electrode can be dedicated to voltage recording, the other to current injection. This is the preferred method, but simultaneous current injection and voltage recording through a single ME can be achieved - one needs a way of eliminating the potential difference between ME barrel and ME tip caused by the voltage drop due to current flowing through the ME

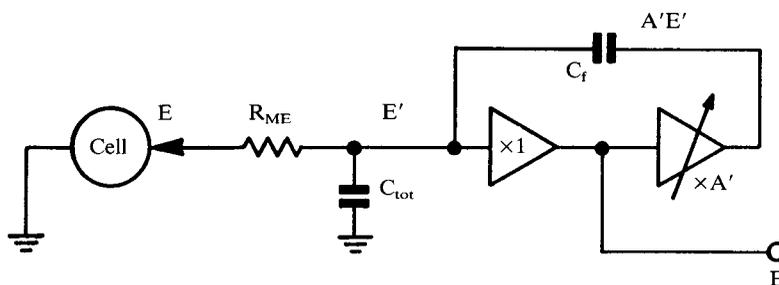


Fig. 3. The potential across C_f is $A'E' - E' = E'(A' - 1)$ and the current across C_f is $C_f \times de/dt \times (A' - 1)$. To compensate for the current loss across the capacitance to ground when E' is changing with time ($C_{tot} \times dE'/dt$) the gain of amplifier A' is adjusted so that $C_f (A' - 1) = C_{tot}$. In this way current is supplied to the input of the amplifier, equal and opposite to the loss through C_{tot} . Another way of regarding this is that of adding *negative capacitance* in parallel with C_{tot} . The effectiveness of a negative capacitance circuit is such that the fully compensated rise time of a circuit is approximately twice the geometric mean of the amplifier's rise time and the uncompensated circuit's rise time (Purves, 1981).

resistance (R_{ME}). These two potentials can be large (i.e. 1 nA through a 50 M Ω electrode = 50 mV). Two methods are available for accomplishing this.

Bridge balance circuits

The modern analogues of the Wheatstone bridge circuitry formerly used for eliminating $E=IR_{ME}$ are still known as bridge balance circuits, though they are not strictly bridge circuits. Nowadays it is usual to subtract electronically from the voltage output a scaled proportion of the input signal driving the current injection circuit (current pump). The circuit in Fig. 4 does just this.

The amplifier gains can be scaled so that the ME resistance can be read off the dial of the BAL potentiometer in M Ω , for example; this is one simple way to measure ME resistance.

Possible artefacts arise from incorrect balancing or the inability to balance the bridge with certainty. First, the method depends on R_{ME} not changing during passage of current through the ME. One should, if possible, use MEs with linear current-voltage (I-V) relationships, or restrict the current to a range of values over which the I-V relation is more or less linear. Second, one cannot compensate completely for the capacitance distributed in the transmural elements comprising the ME tip (C_t). In poor recording conditions, for example deep immersion of the ME in the bath, the frequency response of the recording system is compromised to such an extent that the charging of the cell membrane capacitance cannot be accurately judged (Fig. 5).

Discontinuous current injection method

This is an alternative to bridge balance and eliminates one source of artefact - that of non-linearity of the ME I-V relation. Instead of injecting continuous current, pulses are injected (see Wilson & Goldner, 1975).

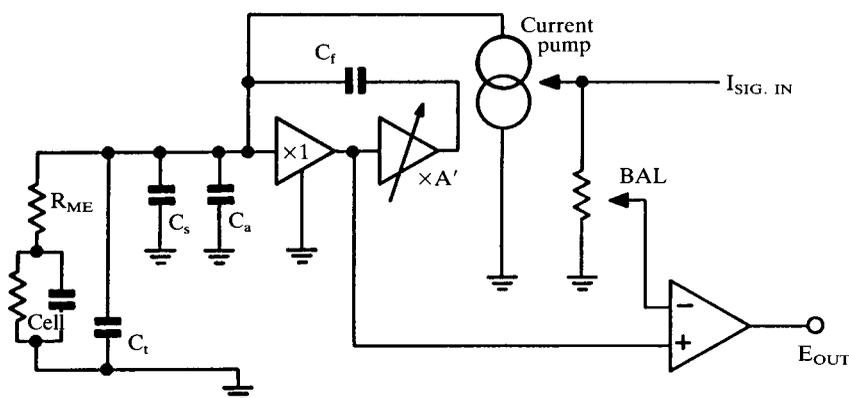


Fig. 4. A circuit to compensate for the potential drop across a ME due to passage of current. The current pump will pass a current proportional to the command voltage and independent of the electrode resistance. The command voltage is therefore scaled and subtracted from the ME potential. The circuit assumes that the ME has a linear current-voltage relation (i.e. obeys Ohm's law). This may not always be true, and should be checked.

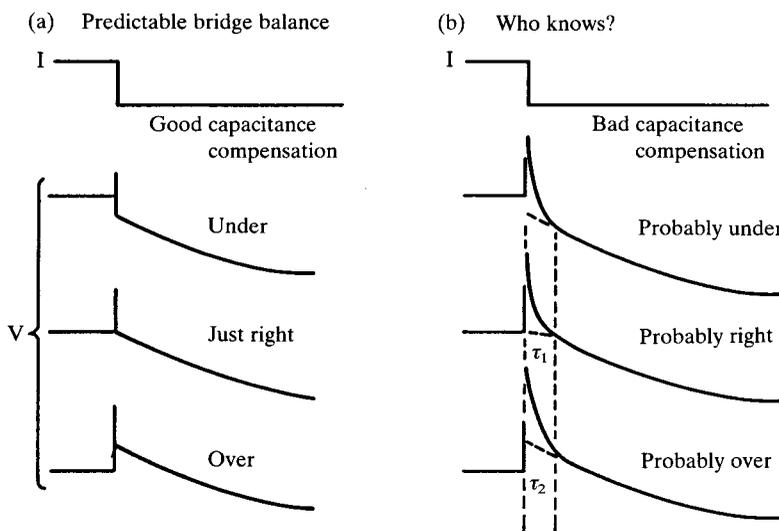


Fig. 5. Incorrect capacitance compensation can lead to spurious balance of the ME resistance.

Fig. 6 shows the principle of the single-electrode switched current clamp. If the membrane time constant is large compared to the electrode time constant, charge will be stored on the membrane capacitance (time constant $C_m \times R_m$), whereas the potential due to IR_{ME} (time constant $C_{tot} \times R_{ME}$) decays rapidly. If the voltage is sampled and held between time points S ($V_{S\&H}$) at a time when IR_{ME} is zero, only V_m , the true membrane potential is measured and the change in V_m approximates that which would be seen in the normal bridge balance case with a continuous current of one half the amplitude (Fig. 6B). Obviously, the approximation is better the higher the pulse frequency. The voltage due to current passage down the ME does not appear and furthermore, since one monitors voltage after IR_{ME} has decayed, any change in R_{ME} during current passage is of no consequence, provided that the current pump can deliver a truly constant current. Clearly, for rapid switching rates capacity neutralization is important. In addition, the noncompensated C_i must be minimized to allow fast settling. Artefacts encountered with this method of 'balancing' electrode resistance stem largely from inadequate capacity compensation or using too high a pulse frequency. These are illustrated in Fig. 7.

If the capacity compensation is correctly adjusted and the ME is clearly settling within a half cycle time then the $V_{S\&H}$ (sample and hold) should exhibit no step with the onset of current injection. To help settling times asymmetric duty cycles may be employed (e.g. 25 % current injection, 75 % settling time). In these cases the current I , delivered with a duty cycle of D is equivalent to a constant current of $I \times D$.

9. Mechanical stability

The interaction between ME (or patch pipette) and cell needs to be stable on a

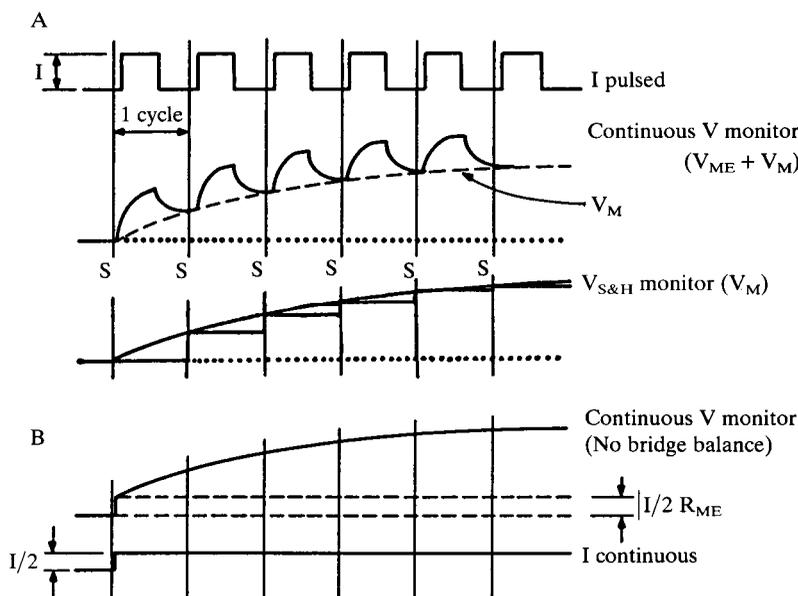


Fig. 6. The principle of the single-electrode switched current clamp is shown. In A, current pulses lead to rapid charging of the ME RC network. The slower charging of the cell membrane RC network leads to a smaller signal which is sampled some time after the end of a current pulse when the charge on RCME has decayed. B illustrates the point that the pulsed current is equivalent to a continuous current of half the pulse amplitude which (a) causes a voltage drop across RME (b) charges the membrane capacitance.

submicron scale to avoid damage to the cell membrane and consequent excessive current leakage. There are two symptoms of mechanical problems, slow drift of the ME tip with respect to cell, and faster vibrations.

Drift can arise from the following causes.

(1) The cell or tissue may move in the bath, a problem that can usually be simply remedied (except when recording from muscle). The bath may move with respect to the microscope stage, remedied by servicing the ratchet drives of the stage movement or by adding locking screws or some other device.

(2) The ME may move in its holder, either axially or laterally, particularly if pressure is applied for injection purposes. Movement of the holder with respect to the micromanipulator can occur with plastic push-in arrangements onto headstage connectors because of 'creep' in the plastic itself. The best arrangement is to secure the ME holder to the micromanipulator directly.

Drift in the micromanipulator can occur between slider assemblies, for example loose rack and pinion drives, screw drives, bearing races or dovetails. If the manipulator is not mounted with flat surfaces then drift can occur due to rocking movements. If the manipulator uses hydraulic drives these are subject to drift because of thermally induced expansion of the fluid, so in this case temperature stability is important.

Vibration of the ME tip with respect to the cell can be minimised by the following

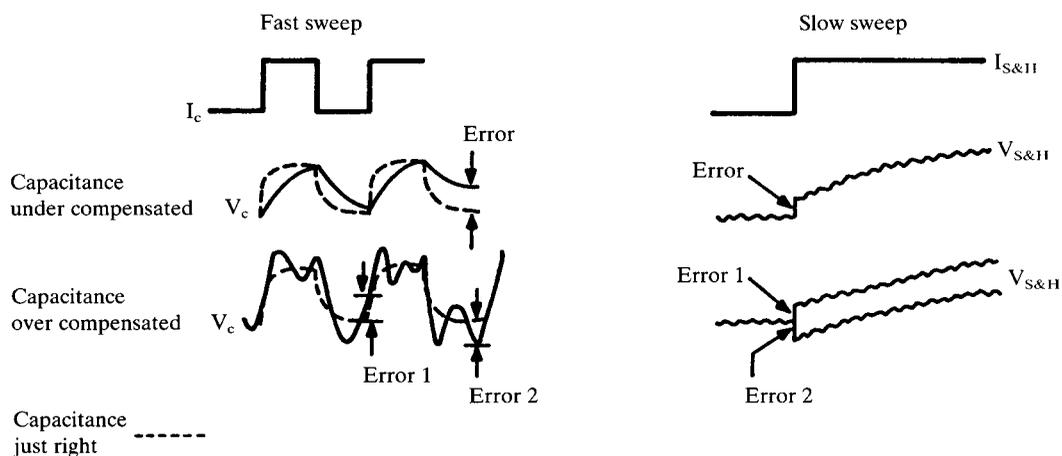


Fig. 7. Artefacts which can arise from inappropriate use of the switched clamp. (I_c , V_c are continuous monitor outputs; $I_{S\&H}$, $V_{S\&H}$ are sampled outputs.)

precautions. The source of vibration is usually floorborne and is eliminated by working on a solid floor, as close as possible to solid ground or at least a supporting wall. Wooden or other unstable flooring can be avoided by sitting the baseplate assembly on brackets bolted to a supporting wall (a cheaper and more effective remedy than an airtable). Vibration reaching the baseplate supports can be filtered by compliant mounts, such as cycle tubes, pneumatic cushions (supplied by optical manufacturers) or regulated compressed air isolation tables, with a heavy table top (total mass 200-500 kg). Generally low frequencies are transmitted more effectively than high through this arrangement; roughly the product of mass and compliance determines the high frequency cut-off. There are two important points to note, that lateral vibration may be as prominent as vertical, and that isolation tables are tuned to a low frequency, 1-2 Hz, to achieve damping and may exaggerate inputs in that range.

The combined transmitting microscope stage - baseplate - micromanipulator - ME holder - ME connections make a system of levers with great potential for vibration, or transmitting small movements to the ME-cell impalement. The connections from the fulcrum of the micromanipulator to the cell should be as short, stiff and light as is feasible. Lightweight eg piezo-driven manipulators mounted on the stage may be the best arrangement, otherwise more remote mounting requires manipulators of massive construction to achieve stability and precision, such as the Huxley design. Vibration in steel baseplates should be damped by bonding to laminated or compressed board and flexion avoided by using sufficient thickness or honeycomb designs.

Micromanipulators should provide precision of movement, much less than 1 μm , with stability in the same range. This is achieved by either lightweight design moving short distances mounted close to the cell, on the microscope or stage, or by more substantial units mounted on the baseplate. In the former category piezo drives or hydraulic drives may provide fine movement of limited range mounted on compact

optical translation stages. This arrangement has the advantage of remote operation of fine movement. In the second category, micrometer or screw drives acting via reducing levers may provide fine movement in only vertical or, in the Huxley design, all three axes. Baseplate mounting of a coarse unit with a remotely operated fine control is often used.

Detection and diagnosis of vibration and slow drift over μm requires a good experimental microscope with graticule eyepiece or TV system.

10. Microscopy

The experimental microscope is an important part of an ME or patch clamp system, often an essential mechanical component as well as a means for seeing the cell. There are a number of books on the workings of the optical microscope, eg by Bradbury, by Ploem & Tanke, and by Spencer.

Generally, ME (and patch clamp) technique is improved by seeing the tip and cell clearly during impalement or seal formation, and there are a number of examples of significant advances in the application of ME measurements resulting from better optics. The iontophoretic studies of postsynaptic mechanism at the skeletal endplate (McMahan *et al.* 1971; Kuffler & Yoshikama, 1975) and autonomic ganglia (McMahon & Kuffler, 1971; Lichtman, 1977) used Nomarski differential interference contrast (DIC) optics to see synaptic elements clearly. The same optical system with infrared video microscopy can be used to see and record from soma or dendritic elements in brain slices (Stuart *et al.* 1993). DIC and fluorescence microscopy are used to identify cell types and developmental stages when investigating changes in electrophysiological properties (see chapter on dye injection and cell labelling). The use of indicators for intracellular ion concentrations requires good optics for fluorescence microscopy (see chapter on fluorescent indicators).

Some general considerations are given here. Good optical resolution requires a good microscope objective, particularly as high a numerical aperture (NA) as is practicable. 'Resolving power', the separation of 2 distinct points, is proportional to $1/\text{NA}$. The practical restriction in an upright microscope is mechanical, because high NA is achieved by decreasing the distance between objective lens and specimen. An upright microscope is essential for work in thick specimens such as tissue slices or embryos and the minimum working distance for placing MEs is about 1.5 mm. The best NA currently available with this working distance is 0.75 in a 40 \times water immersion objective (Zeiss 0.75 W 40 \times), although water immersion objectives exist with NA 0.9 or 1 but shorter working distance¹. For single cells dispersed or in culture an inverted microscope can be used. If the chamber base is made of a thin coverslip working distance is no longer a problem (the ME or pipette comes from above) and the maximum NA obtainable with an oil immersion objective, 1.3-1.4,

1. Zeiss have recently introduced a 63 \times 0.9 NA W 1.4 mm w.d. objective in their infinity corrected range, but this has not been evaluated at time of press.

can be used. Illumination of the specimen should also be with as high an NA condenser as possible; the same restriction of working distance means that a condenser of NA 0.9 can be used with upright microscopy (good illumination is important in thick specimens) but only 0.5-0.6 with inverted.

Contrast methods such as phase contrast, DIC or Hoffman greatly improve the visibility of thin cells and small structures on large cells, and one or other of these is really necessary. Phase contrast is inexpensive and adequate for single cells, but for thicker specimens DIC optics are favoured. CCD TV cameras are also inexpensive and convenient.

For fluorescence microscopy the brightness of the specimen increases with the NA^2 and decreases with $1/(\text{magnification})^2$, so high NA low magnification objectives are often used for microspectrofluorimetry.

11. Summary

The main sources of error in measuring potential with intracellular microelectrodes should be clear. They are:

- (1) Varying tip potentials of the ME.
- (2) Varying junction potentials.
- (3) Asymmetry of electrode reference potentials and their dependence on salt concentration in the bath solution.
- (4) Inadequate amplifier frequency responses when monitoring fast signals.
- (5) Errors in potential measurement when injecting current because of 'bridge balance' or ME resistance change, or due to too high a switching rate when using the discontinuous current injection method.

Most of what we have discussed in this chapter is available in greater detail in Purves (1981). Purves' book is a good source of reference to more advanced texts.

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