Chapter 16

Microelectrode electronics

DAVID OGDEN

1. Introduction

These notes are intended to provide an introduction to the electronics of microelectrode and patch clamp amplifiers. How much electronics do you need to use a physiological amplifier? Enough to know how much distortion is introduced by the measurement. This means (1) testing the response to an input that simulates the physiological signal, (2) calibrating the gain and frequency response, and (3) knowing the errors that might arise from limited performance. This ‘black box’ approach to instruments is the minimum needed and requires a knowledge of the basic principles of electronic circuits. It is fine until something goes wrong or a special requirement arises which prompts a look inside to see how things work and whether a modification can be made.

It is worthwhile taking a practical course in e.g. medical electronics if one is available and working in the electronics workshop for a period to learn soldering from an expert. For those interested in making circuits, applications are given below of operational amplifiers in circuits which may be useful for signal processing and can be built relatively easily and cheaply. Building operational amplifier circuits is a very good way of learning the basics of electronics - the amplifiers commonly used are inexpensive enough to permit a degree of trial and error and standard printed circuit boards are available.

A knowledge of the properties and jargon of low-pass filters is necessary for survival. These are introduced and their use prior to digitizing data for computer analysis is discussed.

Many topics have not been included and for these, and wider coverage of topics introduced here, a list of books and articles for further reading and reference is appended.

Current flow in resistors and capacitors

Current, units Amps, is the rate at which charge (measured in Coulombs) flows at a point in a circuit. The driving potential, measured in Volts, is the energy of each unit of charge, Joules/Coulomb, and is analogous to pressure in a gas or concentration in solution. The conversion between chemical concentration of charged particles and electrical quantities of charge is by the Faraday, about 96500 Coulombs/mole of univalent ion, so 96.5 nA of current flowing in a solution is carried by a flux of 1 picomole of univalent ions/s.

National Institute for Medical Research, Mill Hill, London NW7 1AA, UK

Resistance and conductance. Charge flows through a wire, a solution or other conductive media by the movement of charged particles, electrons or ions, against resistance imposed by random thermal motion. The reciprocal of resistance is conductance.

Current flow in a resistor is proportional to the voltage applied across the terminals. Resistance is measured as 1 Ohm, Ω = 1 Volt/Amp. Conductance, Amp/Volt, is measured in Siemens, S = 1/Ohm.

Capacitance. Charges accumulate where two conductors are in close contact (a capacitor) and at different voltages. Energy is stored by polarisation of the medium (the dielectric) between the conductors. The charge accumulated is proportional to the voltage applied, and charges move into and out of the conductors when the voltage changes.

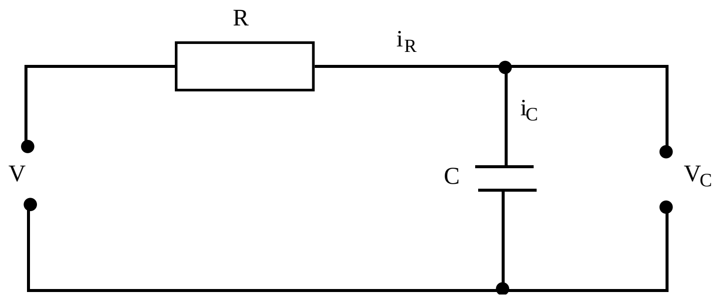
Current flow in a capacitor is proportional to the *rate of change* of voltage, Amps = capacitance × dV/dt. As a consequence the presence of capacitance modifies the timecourse of potential with respect to current flow.

The unit of capacitance measures the accumulation of charge for 1 volt change of potential, 1 Farad = 1 Coulomb/Volt.

Electrical models of the properties of cells and tissues comprise networks of resistors and capacitors, the former representing paths for current along the core and through ion channels in the surface membrane and the latter capacitative flow across the nonconducting lipid bilayer. No charges (ions) physically cross the membrane capacitor, but flow in the adjacent solution as the membrane potential fluctuates.

Time course of capacitor charging. The single most important circuit for an electrophysiologist is the charging, or discharge, of a capacitor through a resistor. For a voltage V applied to a resistor and capacitor in series, the voltage measured across the capacitor, V_C, can be derived as follows.

Assume (1) that the applied voltage can supply enough current (i.e. has negligable internal resistance) and (2) that the voltage measurement draws no current from the circuit.



Current flowing into the capacitor is supplied via the resistor, so currents flowing *into* the junction (defined positive) of R and C, where the voltage across the capacitor is measured, are

$$i_C + i_R = 0.$$

The currents (1) through the resistor and (2) into the capacitor are

$$(1) \qquad i_R = (V - V_C)/R \text{ and}$$

$$(2) \qquad i_C = -C.dV_C/dt$$

(NB. i_C flows into the junction for dV/dt negative)

$$(V - V_C) - R.C.dV_C/dt = 0.$$

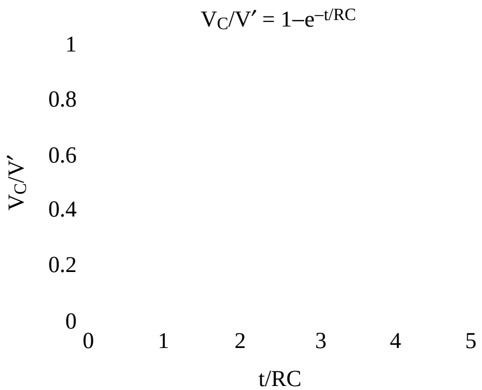
If V changes abruptly from 0 to V′ at time t = 0 then the solution for the time course of V_C is

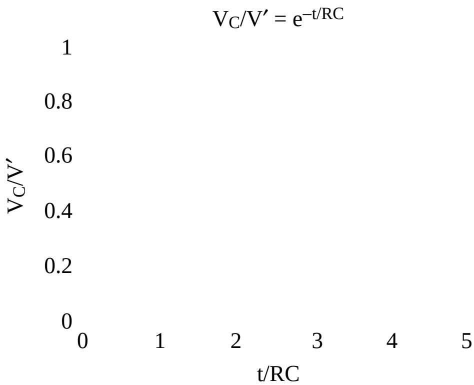
$$V_C = V'(1 - e^{-t/R.C})$$

a rising exponential with final value $V_C = V'$. For the reverse change of V to 0, so V_C discharges from V′ to 0 the timecourse is

$$V_C = V'.e^{-t/R.C}.$$

Both the timecourse of charging and discharge are determined by the product of the resistance and capacitance and this product is known as the *time constant* of the circuit. It has dimensions of time (Ohms . Farads = seconds) and is the time taken to discharge to e^{-1} = 0.38 of the initial value or charge to $(1–e^{-1})$ = 0.62 of the final value.





Ideal circuit elements

It is useful initially to consider circuit elements with perfect properties and to take account of practical limitations or secondary properties at a more detailed level of design, analysis or testing. As an example, the electrical properties of microelectrodes for some purposes may be represented electrically as a resistance, but have capacitance, an inherent tip potential and generate noise as well, all of which may be important under some conditions.

Resistors

Generally, current in resistors is simply proportional to potential difference (1 Ohm, Ω = 1 Volt/1 Amp, V/A).

However, there are important practical considerations :

(1) large currents generate heat ($P = I^2R$ Watts). Most resistors are rated at 0.25 or 0.5 Watt.

(2) Resistors have capacitance across the terminals (~0.1-1 pF) which may be important with high resistances (>10 MΩ) and fast voltage changes (e.g. a step of potential) because current will flow through the capacitance as the voltage changes quickly to its new value, producing an initial spike of current.

(3) Voltage noise in resistors has a component (Johnson noise) which increases with the value of resistance, plus an additional component that depends on the resistor composition and voltage difference applied. The rms (standard deviation) of Johnson noise is $V(rms)=(4kTf_cR)^{0.5}$ (k is Boltzmanns constant 1.36×10^{-9} Joule/degree, T temperature °Kelvin, R resistance, Ω, and f_c the bandwidth, Hz).

(4) Moisture or dirt/grease may conduct appreciable current across high value resistors (>100 MΩ).

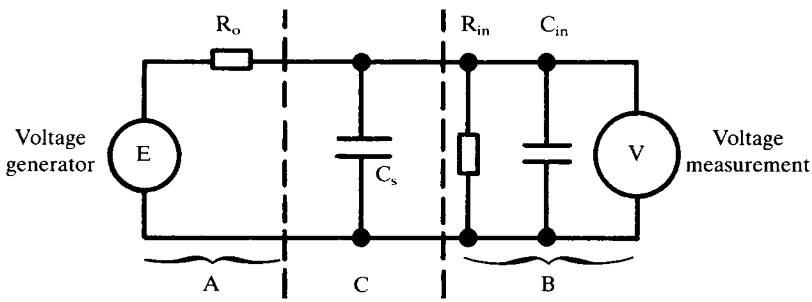
(5) Commonly, resistor tolerances are 5% or 2% - more precise values can be selected (with a digital multimeter, DMM) or obtained with 2 resistors in series or parallel.

Capacitors

The charge, Q (Coulombs), accumulated on the plates of a capacitor is proportional to the potential difference, V, between the plates, Q=C.V, where the capacitance C has dimensions of Farads (F). The energy difference between the plates due to the potential difference (Volts=Joules/Coulomb) is absorbed in the insulating dielectric separating the plates. No current flows between the plates of an ideal capacitor, but if the voltage changes then current, i, flows into the plates as the charge accumulated changes (Amp=Coulomb/s). Practically, some types of capacitor, such as the large value electrolytic types used in power supplies, may have small leakage currents across the plates. Some types distort rapidly changing signals due to the poor properties of the dielectric, and should not be used where fast signals are encountered. Tolerances are usually 20%. Precise measurement of capacitance is much more difficult than the measurement of resistance.

2. Voltage measurement

The circuit below represents the generation and measurement of a potential and can be split into 3 sections:



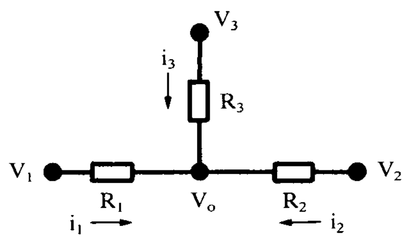
A. *Source of voltage.* The potential V is developed across the output of a voltage generator represented by an ideal voltage source E in series with a small output resistance R_o. Although E produces a constant voltage even if very large currents are generated, the output voltage V is reduced by an amount $(E-V)=IR_o$; this is the situation in real voltage generators, where R_o may represent the internal resistance of a battery or the output impedance of an amplifier. These are normally small (<10 Ω) and only become important at relatively large currents (>100 mA). However, the same considerations apply to microelectrode recording, where E may represent the cell membrane potential and R_o the electrode resistance (> 10 MΩ), so large errors $(E-V)$ may result from small currents (>10 pA).

B. *The measuring circuit* consists of 3 elements, an ideal voltmeter, which draws no current from the circuit and responds instantly to potential changes at the input, a resistance R_{in} to account for current flowing in the input in response to the input voltage, and a capacitance C_{in}. The current drawn by the input is V/R_{in} and determines the error in measuring E, $(E-V) = R_o.V/R_{in}$, so $V/E = R_{in}/(R_o+R_{in})$. The input resistance, R_{in}, of an oscilloscope amplifier is often 1 MΩ or 10 MΩ, of a microelectrode amplifier 10^{12} Ω and of a pH meter 10^{14} Ω. It is clearly important that $R_{in} \gg R_o$ to minimize the error in potential measurements. The input capacitance contributes to slowing the response to a change of V, to an extent that depends on C_{in} and R_{in}; the output for a step input is an exponential of time constant $\tau = C_{in}.R_{in}R_o/(R_{in} + R_o)$ (see below).

C. *The connection* between voltage generator and measuring instrument usually involves a wire of low resistance, but often with important stray capacitance to ground. In the case of screened cable (with braided copper shield connected to earth) this amounts to 100-200 pF/m and may restrict the speed of transmitted signals if it is inseries with a large output impedence. A second effect of this capacitance may be to produce instability in the output of an operational amplifier, requiring insertion of a resistor of 20-50 Ω at R_o to limit the current flowing into the capacitance in fast signals. In the case of a microelectrode or other high resistance source, the stray capacitance should be kept as small as possible by using short connections between the electrode and amplifier input, as discussed below.

3. Rules for circuit analysis

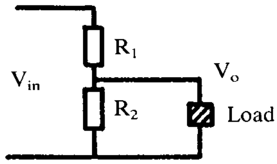
There are two basic rules; (1) currents flowing into a node sum to zero, so in the example shown



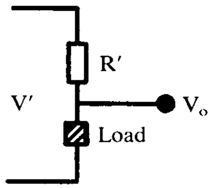
$$i_1 + i_2 + i_3 = 0$$

$$\frac{V_1 - V_0}{R_1} + \frac{V_2 - V_0}{R_2} + \frac{V_3 - V_0}{R_3} = 0$$

(2) the voltage between two nodes is the same *via* all connecting pathways. Complex circuits can often be simplified by applying circuit theory to produce equivalent circuits for analysis. As an example, the circuit

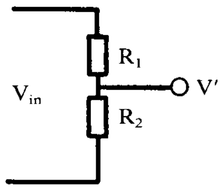


can be simplified to



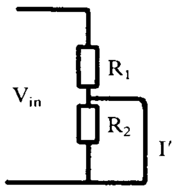
Equivalent

The value of R′ is given by the ratio of the open circuit voltage (i.e. load disconnected), V′, to the short circuit current (i.e. zero resistance load) I′. Thus



Open circuit

$$V' = V_{in} \frac{R_2}{R_1 + R_2}$$



Short circuit

$$I' = V_{in}/R_1$$

and

$$R' = V'/I' = \frac{R_1 R_2}{R_1 + R_2}$$

For example, in the circuit used to describe voltage generation and measurement above, the load can be represented by the parallel capacitors $C_{in}+C_s=C$. The equivalent potential and resistance are given by

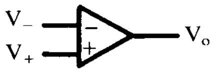
$$V' = E\frac{R_{in}}{R_{in}+R_o} \quad \text{and} \quad R' = \frac{R_{in}R_o}{R_{in}+R_o}$$

and the charging time constant by $\tau=R'C$. The time course of the potential measured, V(t), following a step change of E at t=0, is given by

$$V(t) = V'[1 - e^{-t/R'C}]$$

4. Operational amplifiers

These provide a convenient and inexpensive means of processing analogue signals and may also be suitable for use as input stages, in voltage clamp amplifiers and for current generation and measurement. The most common type has two ‘differential’ (i.e. A–B) inputs and a single output and is represented by

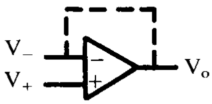


$$V_o = A\,(V_+ - V_-)$$

where (+) is the *non-inverting* input, i.e. the output has the same polarity as (+), and (–) is the *inverting* input, for which the output has opposite polarity. The output voltage is proportional to the difference, $(V_+–V_-)$, of the input voltages. A few microvolts potential difference between the inputs is sufficient to cause the output to change by several volts, so the proportionality constant, or *open loop gain* A, is very large, typically more than 10^5.

Negative feedback

The effect of connecting the output to (–), to produce negative feedback, is to minimize the voltage difference between (–) and (+) inputs as follows.



$$V_- = V_o$$

If the open loop gain is A, then $V_O=A(V_+–V_-)$ and, since $V_-=V_O$, $V_O=V_+A/(1+A)$. Therefore, if A is large, $V_O\approx V_+$ and $V_-\approx V_+$, i.e. to a good approximation the output follows the input, V_+, and voltages on (+) and (–) are equal. By applying only a proportion, say $^1/_x$, of V_O to V_-, a circuit with gain=x is produced. In this case $V_O=A(V_+–V_O/x)$ which can be rearranged to give

$$V_O = xV_+/(1 + x/A)$$

so, provided x/A is much smaller than 1,

$$V_O \approx xV_+ \text{ and } V_- = V_+A/(x + A) \approx V_+$$

In this way, operational amplifiers can be used to give amplification of differing characteristics by modifying the feedback from V_O to V_-.

This kind of analysis can be applied to voltage clamp circuits, in which V_+ is the command potential and V_- the output of the amplifier which monitors membrane potential; the clamp amplifier works to make these equal with gains of 500-5000. However, in this case the cell, the microelectrodes and membrane potential amplifier are included in the negative feedback circuit so the factor x is a complex function of frequency and may become large, particularly at high frequencies, producing poor voltage control and instability.

Amplifier circuits

An approach to building circuits is to suppose initially that amplifiers have ideal characteristics as follows:

(1) Infinite gain ($A>10^6$) so that circuit gain can be set by external components.

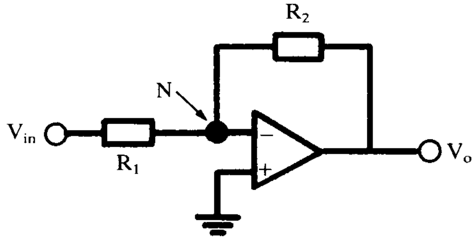
(2) Very high input impedence, so that current flow into the inputs is negligible.

(3) Wide frequency response with no phase changes.

(4) Very low output impedence.

(5) Zero voltage and current offsets at the inputs, so zero input voltage gives zero output voltage. Some basic circuits will be introduced with these properties in mind before considering the deviations from ideal behaviour usually encountered. The two basic configurations have the input signal applied either to the inverting input or to the non-inverting input. In each circuit shown current flowing into the nodes N sums to zero.

Inverting amplifier



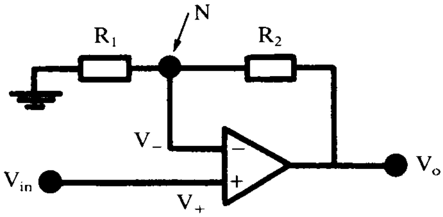
1. $V_- = V_+ = 0$

2. $$\frac{V_{in} - V_-}{R_1} + \frac{V_o - V_-}{R_2} = 0$$

Rearranging and substituting for V_-

$$\frac{V_o}{V_{in}} = \frac{-R_2}{R_1}$$

Non-inverting amplifier



1. $V_+ = V_- = V_{in}$

2. $$\frac{0 - V_-}{R_1} + \frac{V_o - V_-}{R_2} = 0$$

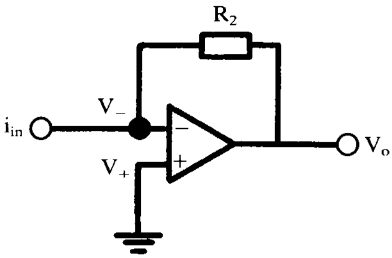
$$\frac{V_o}{V_{in}} = 1 + (R_2/R_1)$$

The gains V_o/V_{in} of these two circuits were obtained in two steps

(1) $V_+=V_-$ i.e. open loop gain A is large.

(2) Sum of currents into the node N is zero with none entering the amplifier inputs.

A number of useful circuits stem from the inverting amplifier. The point V_- is known as virtual ground since it is at the same potential as V_+ i.e. 0 V in the illustration (provided A is large) and the input resistance seen by the signal at V_{in} is R_1. If R_1=0 then a *current to voltage amplifier* results since the input current is equal to the feedback current ie. $-V_O/R_2$.



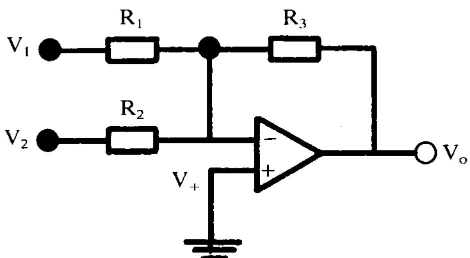
1. $V_- = V_+ = 0$

2. $$\frac{V_o}{R_2} + i_{in} = 0$$

$i_{in} = -V_o/R_2$

Summing amplifier

A number of inputs may be summed with differing gains as follows.



1. $V_+ = V_- = 0$

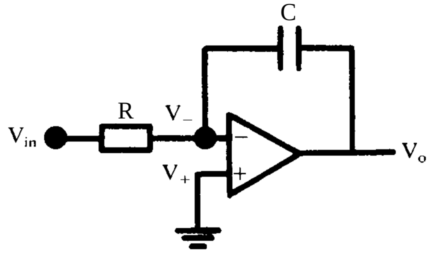
$$2.\quad \frac{V_1}{R_1}+\frac{V_2}{R_2}+\frac{V_o}{R_3}=0$$

$$V_o=-V_1\frac{R_3}{R_1}-V_2\frac{R_3}{R_2}$$

$$\text{Gain for input } V_1\ \frac{V_o}{V_1}=-\frac{R_3}{R_1};\ \text{for } V_2\ \frac{V_o}{V_2}=-\frac{R_3}{R_2}$$

Integrator

The mean level of the input voltage over time may be estimated by integration with a capacitor as the feedback element.



$$1.\quad V_-=V_+=0$$

$$2.\quad \frac{V_{in}}{R}+C\frac{dV_o}{dt}=0$$

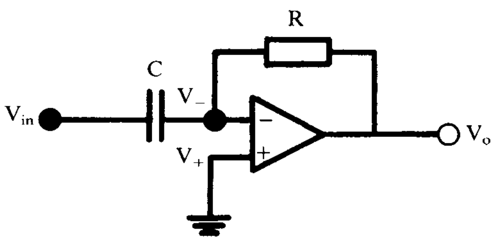
Rearranging and integrating from 0 to t gives

$$V_o(t)=\frac{-1}{RC}\int_o^t V_{in}\,dt$$

Integrators usually require a variable steady offset voltage at V_1 to zero the input initially and a 'reset' switch to discharge C to zero at the end of a measurement.

Differentiator

The first time derivative of a signal may be obtained by reversing the positions of R and C.



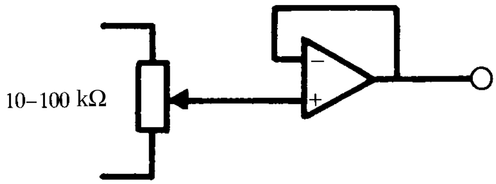
$$1.\quad V_-=V_+=0$$

$$2. \quad \frac{CdV_{in}}{dt} + \frac{V_o}{R} = 0$$

$$V_o = -RC\frac{dV_{in}}{dt}$$

Non-inverting amplifiers

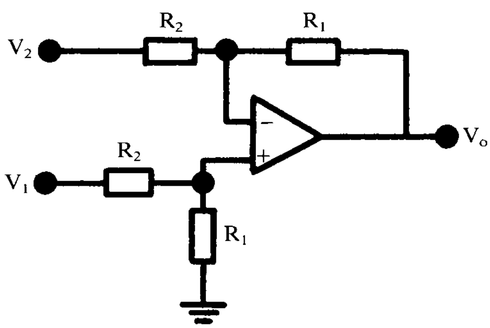
These are used mainly as buffers from a high resistance voltage scource, e.g. a potentiometer, to provide a low output resistance to drive the subsequent circuitry. The most common gain used is 1, i.e. as a voltage follower



but gains of 10 or more may be used as described above. If good quality operational amplifiers are used, the voltage follower configuration may be used in microelectrode amplifiers.

Differential amplifiers

In this case signals are applied at both (+) and (–) inputs and the difference signal V_1–V_2 is required, sometimes with a gain factor. The following circuit uses a single operational amplifier, although for fine tuning of gain and rejection of signals common to both V_1 and V_2 ('common mode rejection'), a circuit with two or more amplifiers may be preferable.



$$1. \quad V_+ = V_1\frac{R_1}{R_1 + R_2}$$

$$2. \quad \frac{V_2 - V_-}{R_2} + \frac{V_o - V_-}{R_1} = 0$$

Since $V_- = V_+$, rearranging gives $V_o = \dfrac{R_1(V_1 - V_2)}{R_2}$

It can be seen that the gain and common mode rejection (the V_o obtained with V_1=V_2) of this circuit requires accurately matched values of resistors R_1 and of resistors R_2.

Non-ideal characteristics

(1) The open loop gain (A) is high at low frequencies, usually about 10^5 at 0 Hz, but declines markedly as frequency increases, falling to 1 at about 10^5-10^7 Hz, depending on the amplifier. The product of gain and bandwidth is a characteristic sometimes specified. Good frequency response can only be obtained at low circuit gain, x, since the condition A/x»1 has to be maintained over a wide frequency range. Gain-bandwidth product is often 100 kHz-1 MHz but may be lower e.g. the 'standard' 741 has only 10 kHz. It is usually better to realize high gains with 2 or more sequential amplifiers of low («100) gain if good frequency response is required.

(2) Stability. Phase changes occur at high frequencies which may result in instability due to positive feedback of these frequencies at gains >1. External compensation or a small feedback capacitor may be required to reduce the gain at high frequencies.

(3) Input resistance and leakage currents. The input impedence and leakage currents depend on the type of transistor junction used at the input. Bipolar inputs have 1-2 MΩ impedence and 0.1-10 nA leakage current. The μA741 and NE5534 are commonly used bipolar types. Junction field effect transistor (JFET) inputs may have 10^{11}-10^{13} Ω impedence and 1-100 pA leakage current e.g. LF356, BB3523. Other inputs e.g. MOSFET or varactor diode inputs may have 10^{13}-10^{15} Ω impedence.

(4) Offset voltage of 0.5-2 mV referred to the input is usually present and can usually be adjusted by an external potentiometer to give zero output.

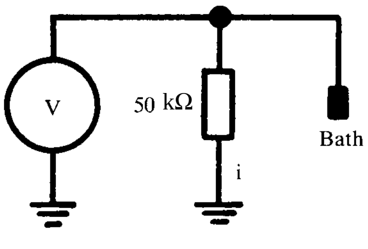
(5) Noise and drift are usually acceptable but if a critical application is needed, such as a microelectrode input stage, more expensive versions of standard amplifiers selected for low noise and low drift should be used.

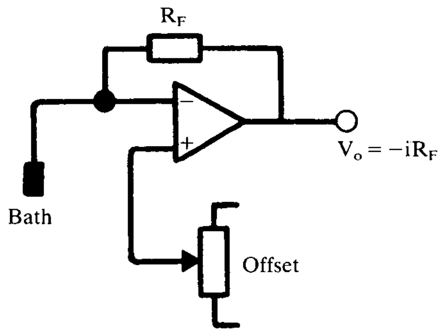
Some practical suggestions

It is straightforward and inexpensive to make basic circuits for signal processing, even if some integrated circuits are destroyed in the process. If possible, use preformed printed circuit boards, e.g. from RS™, which reduce the possibility of wiring errors. Always 'decouple' each amplifier from interference in the supply lines by 1-10 μF tantalum (NB polarity of tantalum capacitors) and 0.1 μF ceramic capacitors (for removing high frequency interference) from +15 V and −15 V to the common of the power supply. Use a terminating resistor of 50-100 Ω on the output if a screened cable is used. Make sure that the ground lines to the input and feedback circuits of the amplifier do not carry currents to the common of the power supply, or other sources of large currents, by use of a parallel earthing pattern to a common point.

5. Current measurement

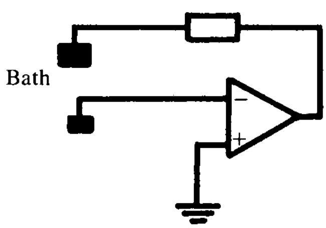
Current flow in a circuit is measured as the voltage difference across a known value resistor. In microelectrode experiments the current to ground from the preparation bath is often required. Measurement by the voltage across a resistor e.g. 50 kΩ connected in the ground from the bath is unsatisfactory, mainly because the potential of the bath then varies with the current and the sensitivity is low (50 μV/nA).





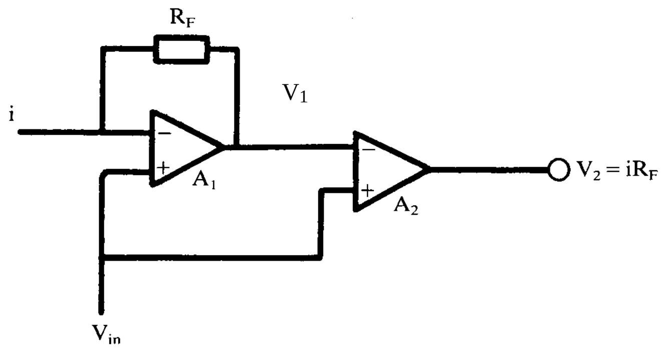
A better arrangement is the virtual earth circuit discussed above, which has the advantage that the bath potential is clamped at a constant level (set by the offset circuit), provided currents are not so large that polarization of the bath electrode occurs. The sensitivity is now set by the value of R_F, $V_O=-iR_F$, without affecting the bath potential, and practically may be 1 or 10 mV/nA (R_F=1 MΩ or 10 MΩ).

A second use of the virtual ground circuit is to avoid changes of bath potential resulting from polarization of the ground electrode with large current flow (>1 mA). The current is supplied via the feedback arm to a large surface area platinum or AgCl electrode and the bath potential monitored with zero current flow at the inverting input by means of a stable AgCl electrode. It should be noted that the bath electrodes form part of the feedback circuit and the output V_O is therefore influenced by polarization and not useful for current measurement directly.



Current measurement within a circuit is also achieved with an inverting amplifier.

A commonly used circuit is

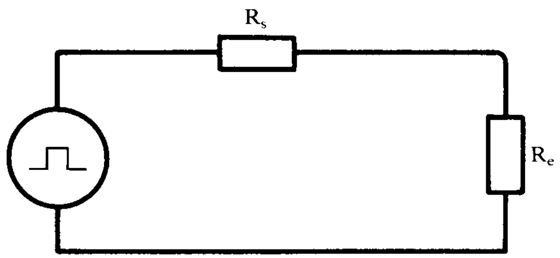


(1) $V_- = V_{in}$ (2) $iR_F = V_{in} - V_1$ (3) $V_2 = V_{in} - V_1 = iR_F$

where the second stage is a differential amplifier (A2, resistors omitted) used to subtract V_{in} from the output of the current to voltage amplifier A1. This arrangement is used in the patch clamp amplifier and sometimes in the current injection and monitoring part of voltage clamp circuits.

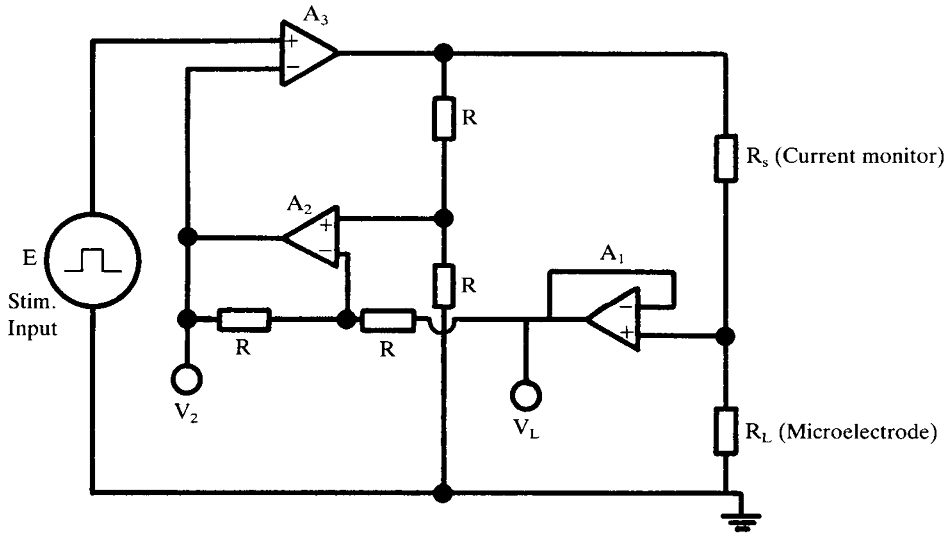
Injection of constant current

A source of constant current pulses is useful for ionophoresis, dye injection and determining the passive electrical properties of cells. A 'constant current source' ideally injects constant current into even a very high and fluctuating load resistance such as a microelectrode. An approximation to this may be achieved with a large voltage (e.g. 100 V) and large resistance (R_s=100 MΩ–1 GΩ) in series with the microelectrode (R_e=10–100 MΩ). In order to give constant current, $R_s \gg R_e$.



This arrangement works for small currents. With large currents, a capacitative transient may occur due to conduction of the rapidly rising edges of a rectangular pulse over the parallel capacitance of the large value series resistor.

Constant current can be generated electronically by clamping the potential across a resistor in the current path by means of operational amplifiers. A schematic diagram of a circuit given by Purves (1981, 1983) is as follows.



$$V_2 = IR_s \quad V_L = IR_L$$

E is the output of a stimulator, and resistor R_S is of known value (10 or 100 Ω) used to monitor current through R_L, which is a variable load (e.g. a microelectrode). Amplifiers A1 and A2 form a differential amplifier of unity gain to monitor the voltage across R_S; A1 acts as a high impedence buffer drawing no current from the injection path through R_S and R_L. V_2, the output of A2, gives the current through R_L as $I=V_2/R_S$. This signal is also compared at A3 with the command E, A3 acting to maintain $V_-=V_+$, keeping V_2=E and therefore I/R_S=E, even if the load resistance, R_L (microelectrode + cell) changes. The potential at the microelectrode R_L can be monitored with the output of A1.

6. Filters

Purpose

Filters are used to remove unwanted high or low frequency components of a signal usually to improve the signal/noise ratio. The most common applications are (1) to remove high frequency noise arising in the recording instruments, microelectrodes or radio interference. (2) To prevent aliasing of digitized signals when sampling into a computer or other digital equipment. (3) To remove the steady DC component and low frequencies when recording low amplitude fast events superimposed on a steady membrane potential or current. It is important to remember that in addition to generating unwanted noise all electronic apparatus has some degree of inbuilt filtering.

Properties of filters and an explanation of filter jargon

Low pass filters progressively reduce the amplitude of high frequency signals; low frequencies below a specified value are passed unattenuated. These are particularly useful in removing high frequency instrument noise from relatively low frequency biological signals.

High pass filters produce attentuation of low frequencies below a specified value. Steady signals give zero amplitude output. The a.c. switch on oscilloscope amplifiers produces high pass filtering below about 1 Hz.

Band pass filters attenuate frequencies above and below specified values.

Filter characteristics are represented (1) in terms of the amplitude response to sine wave inputs of different frequencies and (2) the time course of the response to a step input.

(1) The POWER SPECTRUM of a signal is the power generated in a nominal 1 Ohm resistor at different frequencies plotted against frequency, usually on log-log coordinates. Power spectra are most often plotted as the ratio of output power to input power P_{out}/P_{in}. Fig. 1 shows the power spectrum of white noise (equal power at all frequencies) as a straight line parallel to the abscissa and the outputs of low pass, high pass and band pass filters to a white noise input. These show the attenuation of high (low-pass) or low (high-pass) frequencies as described above.

The HALF POWER or CUT OFF frequencies, f_c, occur where the output power is reduced to 0.5 of the input power. Power ratios are usually given in decibels (dB)

$$dB = 10 \log_{10} P_{out}/P_{in}$$

For $P_{out}/P_{in}=0.5=-3.01$ dB. For this reason half power frequencies are known as -3 dB frequencies when specifying filter properties.

The ratio of output voltage to input voltage V_{out}/V_{in} is more useful than power ratios and filter characteristics are given as log-log plots of V_{out}/V_{in} against frequency. The relation between power and voltage is

$$P = V^2/R$$

so the power ratio is equal to the square of the voltage ratio:

$$P_{out}/P_{in} = (V_{out}/V_{in})^2, \text{ hence } 1 \text{ dB} = 20 \log (V_{out}/V_{in})$$

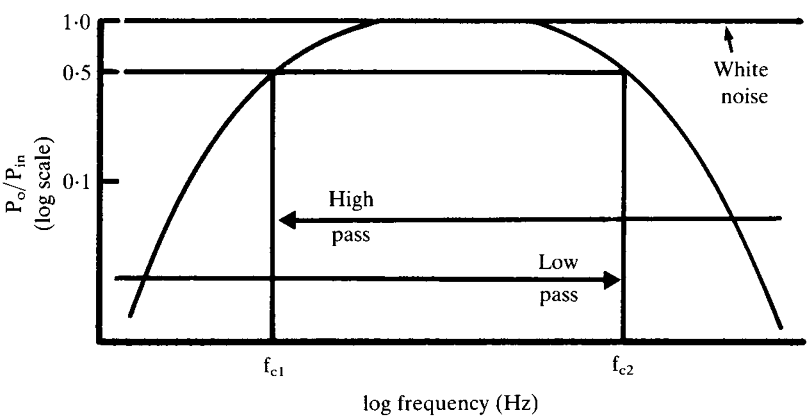


Fig. 1. Power spectrum to show high (f_{c1}) and low (f_{c2}) pass filtering of white noise input.

For

$$P_{out}/P_{in} = 0.5 \quad \text{then } V_{out}/V_{in} = (0.5)^{0.5} = 0.7$$

The halfpower frequency, f_c, of the voltage ratio therefore occurs at $V_{out}/V_{in} = 0.7$, as shown in Fig. 3.

For a low pass filter, the BANDWIDTH is DC (zero frequency) to f_c. For a single stage resistor-capacitor (R-C) low pass filter (Fig. 2) the *power spectrum* at high frequencies has a final slope of –2 on a log-log plot, since the power declines with $1/f^2$. For *voltage* amplitude the log-log plot has a final slope of –1. Both correspond to –6 dB per octave (2 fold frequency change) or 20 dB per decade. A single stage filter of this kind is termed a *single pole* filter. More elaborate higher order filters contain several R-C stages arranged to optimize the roll-off and often have 2, 4, 8, 16 or 32 poles, giving corresponding slopes of –12, –24, –48, –96 or –192 db/octave. The properties of higher order filters are shown in Figs 3 and 4.

(2) The response of a low pass filter to a STEP INPUT of voltage is for most experiments the more important property. The sharp cut-off with frequency achieved with many higher order filters is at the expense of overshoot (or undershoot) of the amplitude following a step, as shown in Fig. 5, and will result in distortion of transients.

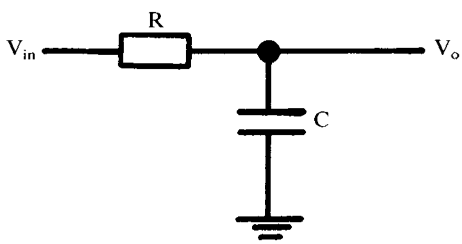
Ideal filter properties

(1) Sharp transition from conducting to non-conducting at $f=f_c$.
(2) Flat frequency response in the pass band ($f < f_c$ for lowpass; $f > f_c$ for high pass).
(3) No distortion of transient or step inputs.

There are 4 basic types of filter response commonly used. The good and bad characteristics of each are compared below and illustrated in Figs 3-5.

Simple R-C filters. Single stage R-C filters are encountered in oscilloscope and chart recorder amplifiers and are easily constructed. Roll-off is poor but can be improved by cascading R-C sections. However, attenuation at frequencies near $f=f_c$ is always poor.

Butterworth characteristic. Response in passband is flat and attenuation at $f=f_c$ is good. Suitable for noise analysis. Produce delay and overshoot in response to



$$V_o/V_{in} = \{1+(f/f_c)^2\}^{-\frac{1}{2}}$$
$$f_c = 1/2\pi RC$$

Fig. 2. Single stage R-C low pass filter.

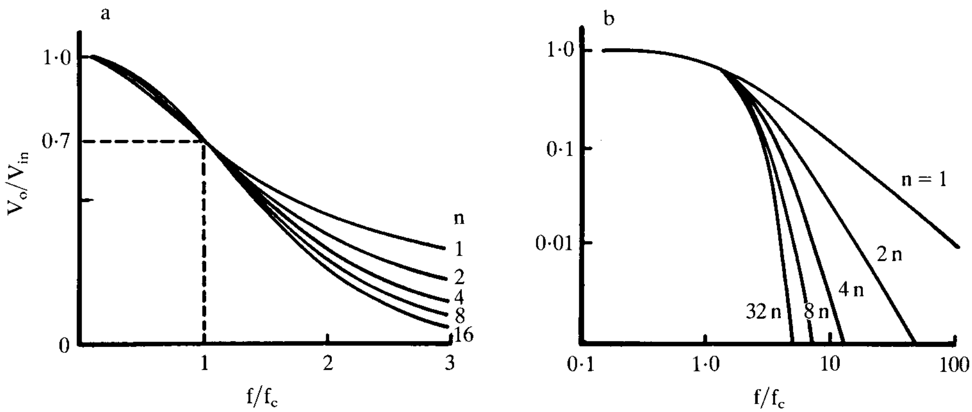


Fig. 3. Cascade R-C filter sections. (a) In the region near f_c on a linear scale. (b) On log coordinates. n is the number of cascaded sections (poles).

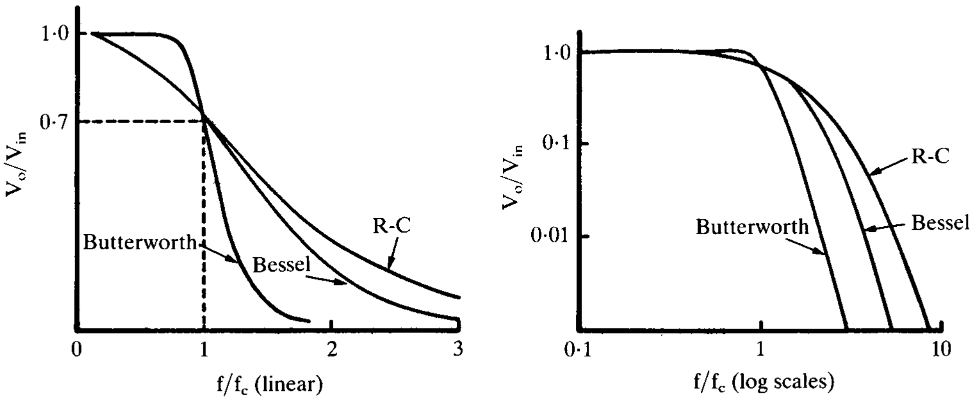


Fig. 4. Comparison of filter characteristics for n=6.

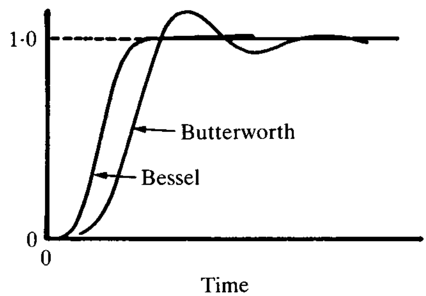


Fig. 5. Response of Butterworth and Bessel low pass filters (n=6) to a step input at t=0.

transient signals, therefore unsuitable for single channel currents, action potentials, synaptic potentials, voltage clamp currents etc.

Bessel characteristic. No delay and minimum overshoot with transient input. Suitable for single channel current etc. signals. Attenuation in region $f=f_c$ is poor and therefore not as good as Butterworth for spectral analysis of noise signals.

Tchebychev characteristic. Good attenuation at $f=f_c$ and a steep roll-off. However, the passband contains some degree of ‘ripple’ and transient inputs are distorted. This type of response is unsuitable for analysis but may be encountered in some equipment e.g. in FM tape recorders to remove the carrier frequency.

Data sampling and digitization

The main application of high order (4, 8, 16 pole) filters is in sampling a signal prior to digitization by a laboratory interface for computer display or analysis. During digitization the amplitude of the signal is measured at constant intervals determined by the sampling frequency, and stored as binary numbers.

Spectral (noise) analysis. The signal can be regarded as a sum of periodic waves of differing frequencies, phase and amplitude. The highest frequency that can be measured will be determined by the sampling frequency. The need to use high order low pass filters arises from the possibility of *aliasing* in the digitized record, i.e. the spurious addition of frequencies higher than 0.5 times the digitizing frequency to frequencies within the range sampled. If f_s is the digitizing frequency and $f \ll 0.5f_s$, then the signal at frequencies of $nf_s \pm f$ (n is an integer) appears added to that at f. To avoid this, the maximum frequency that can be present in the record without producing aliasing is $f_N=0.5\ f_s$ (the Nyquist frequency) so data are low-pass filtered at or below f_N during sampling. An illustration of aliasing is given in Fig. 6, which shows the periodic wave at the Nyquist frequency sampled twice every cycle, and waves of frequencies $(f_N-\Delta f)$ lower and $(f_N+\Delta f)$ higher. If both are present in a signal they are sampled and contribute to the total amplitude. However, their frequencies are indistinguishable at this sampling rate and the sum of their amplitudes would be attributed to $(f_N-\Delta f)$.

Maximum suppression of high frequencies is achieved with a high order Butterworth type response, which is suitable for noise analysis at frequencies up to $f_c=f_N$.

Transients. As mentioned above, the Butterworth response is unsuitable for transient signals. The Bessel response is OK but has poor suppression of frequencies in the region of the half power frequency. The bandwidth should be selected so as not to distort the rise and fall times of the transients. Generally for a Bessel type the 10-90% risetime for a step input is $0.34/f_c$. An action potential rises in less than 100 μs and a bandwidth of 5-10 kHz is needed to avoid distortion. Data should be sampled at 5-10 times the desired bandwidth to ensure good definition of the time course of the transient.

Sequential low pass filtering. Data may be filtered more than once before sampling or final display as a result of low pass filters in the several instruments used for recording, storing and playing back data. The net result is an approximate addition of

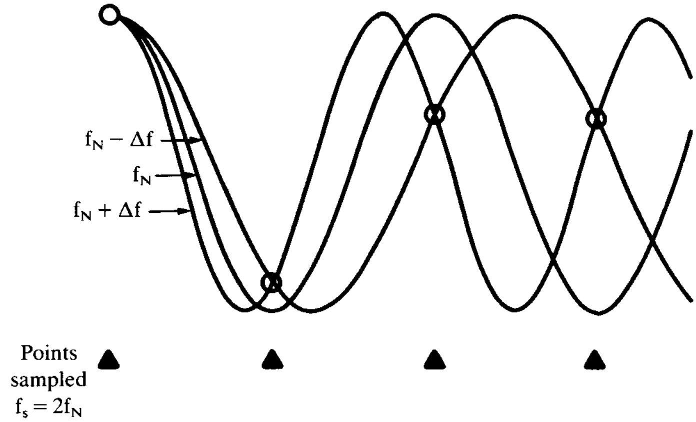


Fig. 6. Aliaising of (fN+Δf) with fN−Δf).

filters so that $1/f^2{}_c=1/f^2{}_1 + {}^1/f^2{}_2$... The most likely source of unexpected filtering is the high order filter of the playback amplifier of F.M. tape recorders.

7. Instruments

It is important to know (1) what is required of an instrument in order to make a particular measurement, (2) to be able to test and calibrate an instrument to see how well it satisfies the requirements, and (3) to build, modify or repair electronic circuits when necessary. These notes will concern voltage (microelectrode) amplifiers and current-to-voltage (patch clamp) amplifiers.

Microelectrode amplifier

It is useful to think of the amplifier as an ‘ideal’ voltmeter (i.e. with none of the following faults) connected to noise generators, input resistance and capacitance as indicated in Fig. 7.

The main requirements for microelectrode recording are as follows:

(1) The current flowing into the input, i.e. from the cell, should be small enough that appreciable redistribution of ions does not occur as a result of the transmembrane flux produced by this current. Also, a potential across the tip of the microelectrode will result, which varies with the electrode resistance. Values <10 pA are alright except for very small cells; values of 1 pA are obtainable with good JFET inputs, but lower values, e.g. for use with high resistance ion-sensitive electrodes, require special input amplifiers. Inputs with ‘bridge’ arrangements to balance out the contribution of the microelectrode resistance during current injection should be carefully checked for leakage at the zero current setting.

The procedure for measuring input current is simply from the change in voltage output on short cicuiting a high value resistor (100 MOhm) connected between input and amplifier ground.

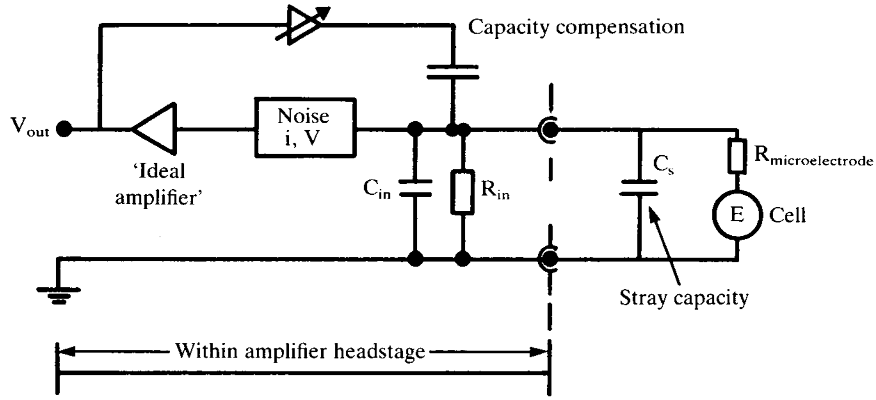


Fig. 7. Schematic diagram of microelectrode amplifier headstage and microelectrode.

(2) The amplifier should have high input resistance (impedance) compared with the microelectrode - the proportion of the membrane potential measured is $R_{in}/(R_{in}+R_{me})$. Most JFET inputs are 10^{12} Ω but varactor amplifiers or MOSFET inputs (10^{15} Ω) are needed for high resistance ion sensitive microelectrodes.

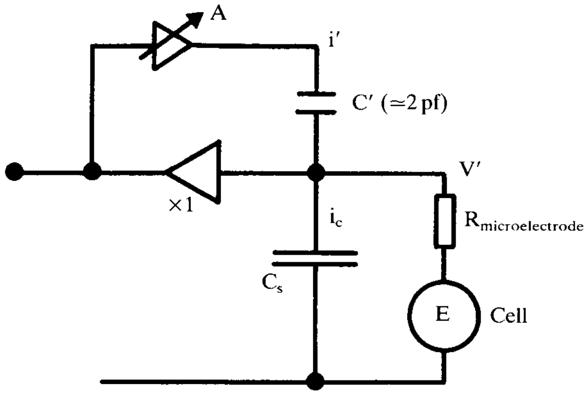
(3) Input noise should be small. Voltage noise with the input grounded is usually about 20 μV peak-peak at dc-10 kHz bandwidth. The major noise source is the microelectrode which contributes Johnson (resistance) noise plus an excess noise arising in the microelectrode, approx. 200 μV p-p for 10 MΩ electrode. Current noise in the amplifier input flows through the microelectrode and may be important for high resistance electrodes-1 pA r.m.s. current noise through a 100 MΩ electrode gives 100 μV r.m.s., about 400 μV p-p. As a result of contributions from these sources, noise increases more than proportionally with microelectrode resistance. This aspect of amplifier performance should be measured with both low and high values of test resistor for comparison with the Johnson noise, given by $V(rms)=(4kTf_cR)^{0.5}$ (k is Boltzmanns constant 1.36×10^{-9} Joule/degree, T temperature °Kelvin, R resistance, Ω, and f_c the bandwidth, Hz).

(4) Response time is usually limited by 'stray' capacitance to ground (C_s) arising from input transistors, connecting wires and across microelectrode walls to bath solution. The response to a rectangular input voltage rises exponentially with time constant $\tau=R_{me}.C_s$ - typical values of 50 MΩ and 10 pF give $\tau=500$ μs ($f_c=1/(2\pi\tau)=320$ Hz). Sources of capacitance are:

(1) between drain and gate of FET input transistors, 3-8 pF. Can be reduced by careful amplifier design (e.g. 'bootstrapping' or cascode input configuration) to 0.1-0.5 pF.

(2) Connecting wires, through proximity to screens. This can be reduced by minimizing length of wires and driving screens with the output voltage of the amplifier (see below).

(3) Capacitance across the microelectrode wall to the bath. This can be reduced by decreasing the depth of fluid in the bath. Painting the electrode with conductive paint,



$i_C = C_S dV'/dt \quad i' = A.C'dV'/dt$
Gain A adjusted for $A.C' = C_S$ to give $i' = i_C$.

Fig. 8. Capacity compensation.

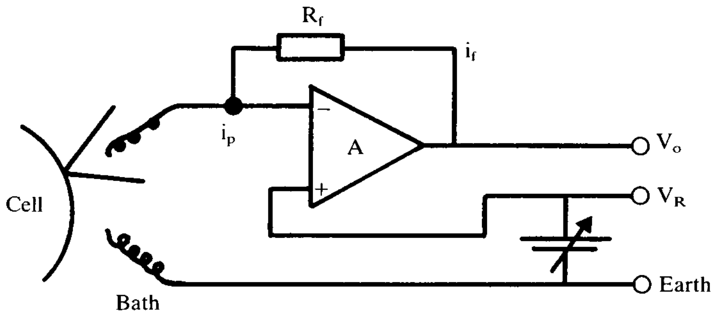
insulating with varnish and driving the paint screen with the ×1 output is very effective. Driven screens of this kind place the same voltage signal on the screen as that present on the input, thereby removing capacitative coupling between input and screen for signals of similar waveform, without loss of shielding from unwanted external scources. However, current noise applied to the screen from the output will be transmitted capacitatively into the input and so may make recordings with high resistance electrodes noisier.

Capacity compensation/neutralization: (see Fig. 8) The current through the stray capacity is compensated with current generated by a variable amplified output (1-10×) applied through a fixed capacitor to the input, generating a current proportional to dV/dt. Problems occur first in adjusting the compensation correctly so as not to distort the input signal, and also from the injection of current noise with the compensating signal. Capacity compensation works best when the stray capacity is initially small, so the precautions aimed at reducing stray capacity cited above are still worthwhile.

Procedures that may be used to test the response time of the microelectrode and amplifier (with or without compensation) are, (1) applying a rectangular voltage to the bath solution with just the tip of the electrode in the solution or (2) applying a triangular pulse through a small value (≈ 1 pF) capacitor into the input. This latter procedure injects a rectangular pulse of current into the input and avoids spurious coupling through the capacitance of the microelectrode wall from the bath solution, which is present with the former method.

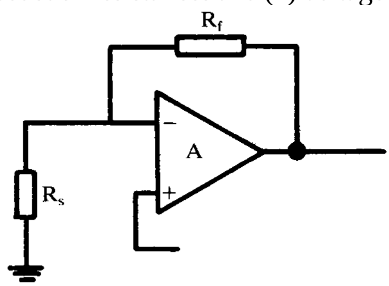
Patch clamp amplifier

The patch clamp amplifier is a current to voltage (I/V) amplifier with basic configuration



where the amplifier A maintains the (–) input at the same potential as (+) i.e. V_R, by negative feedback through resistor R_f. Thus, if the current flowing into (–) is neglected, $i_p+i_f=0$ and $(V_O-V_R)=i_fR_f=-i_pR_f$.

Background noise in patch clamp recording depends critically on the impedence of pipette-cell seal at the input and on the feedback resistance, R_f. It is important to remember that *current* noise at the input matters in patch clamp recording. Sources of noise are (1) the seal and feedback resistances and (2) voltage noise in the amplifier input.



(1) The (–) input is connected to ground via R_s (seal and bath) and R_f (amplifier output). The source (seal) resistance R_s and feedback R_f give rise to voltage noise of approximately

$$V_{rms} = (4kTf_cR)^{\frac{1}{2}}$$

for ideal resistances, where k is Boltzmanns constant, T temperature (Kelvin) and f_c is the upper frequency limit (bandwidth, i.e. low pass filter setting). The current noise

$$i_{rms} = V_{rms}/R,$$

so

$$i_{rms} = (4kTf_c/R)^{\frac{1}{2}}$$

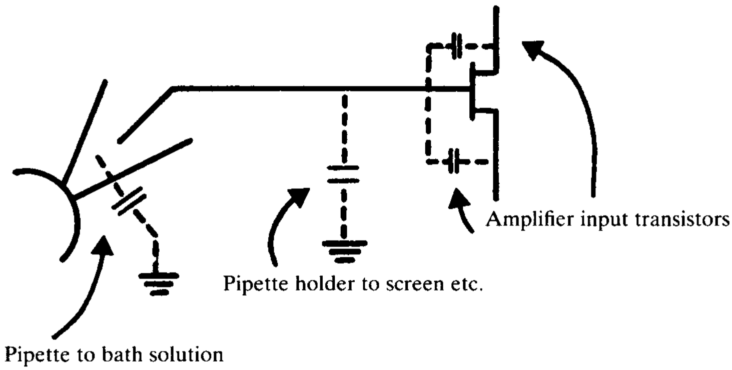
Thus, background current noise due to source and feedback resistances decreases as 1/R. Values of R_f of 10-50 GΩ are used for this reason, and good seal resistances (5-50 GΩ) are necessary for low noise recording (see also next section).

(2) Voltage noise arises in the JFET transistors used for the input stage of the amplifier. This is due to (a) ‘shot noise’ of the input current, resulting from movement of discrete charge carriers (b) thermal variations in internal current flow through the JFET seen as input voltage noise when feedback is applied to the input. This voltage noise gives rise to currents flowing in the stray capacitance of the input. The currents increase greatly with recording frequency such that spectral density of input current

$$S_i = (2\pi f C)^2 . S_V$$

where f is the frequency bandwidth, C the total capacitance and S_V the spectral density of input voltage noise.

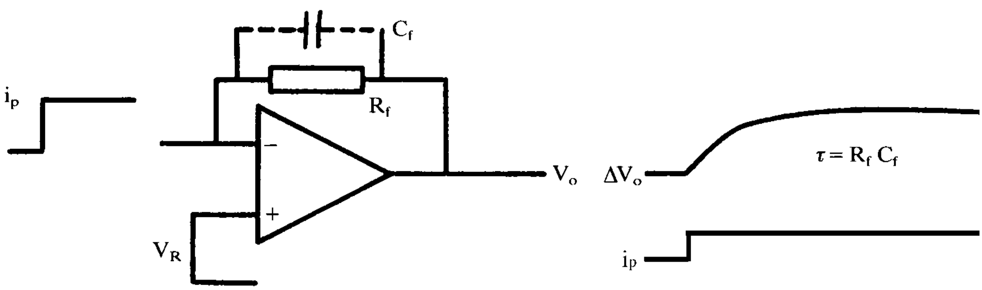
Sources of input capacitance are (1) within the amplifier, mainly across FET junctions and from input lead to ground, approx. 10-20 pF. (2) Across the holder and pipette to adjacent grounded surfaces e.g. microscope and screens. (3) Across the pipette wall to the bath solution. Use of Sylgard resin or other treatments to coat the pipette exterior reduces capacitance considerably by decreasing creep of fluid along the outside of pipette glass and by increasing the thickness of the pipette wall. Minimising the bath level and drying the electrode holder when changing pipettes also reduces capacitance due to fluid films.



Sources of stray capacitance.

For a good amplifier, a coated pipette and a good seal, the contributions of the electronics, the pipette and the seal to the total noise are approximately equal, as indicated in Fig. 9.

Frequency response of patch clamp amplifiers. The large values of feedback resistor used in the patch amplifier result in an output time course, following a sudden or step input current, which is dominated by the parallel stray capacity associated with the resistor. The patch clamp thus has a low-pass filter charactaristic, so a 50 GΩ resistor with capacity across the terminals of only 0.1 pF gives a response time constant of τ=5 ms to a step input. This is too slow.

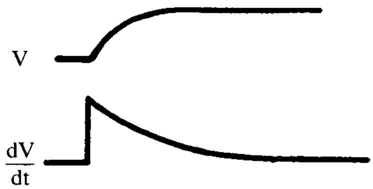


Provided parallel capacity is uniformly distributed over the resistor the response is approximately a single exponential. The output voltage for a step input of current i_p is

$$\Delta V = i_p R_f (1 - e^{-t/\tau})$$

A compensating circuit is employed to correct for this slow response by (a) differentiating the response,

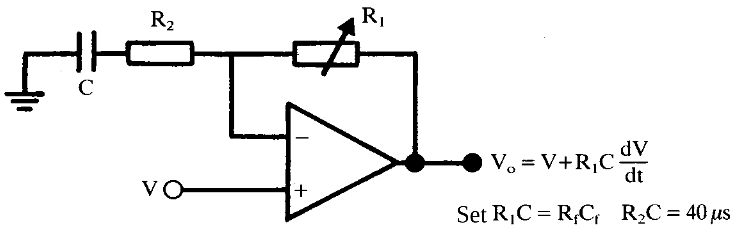
$$\frac{dv}{dt} = \frac{-iR_f}{\tau} e^{-t/\tau}$$



(b) scaling the differentiated response by τ and adding it to the response itself

$$V + \tau dV/dt = -iR_f$$

This procedure is valid for any input waveform and is not affected by pipette capacitance, providing compensation for C_f independent of recording conditions, and can give response time constants of 20-50 μs for a step input. A circuit that performs differentiation, scaling and addition which is often used for compensation is



This operates as a voltage follower at low frequencies but increases in gain at high frequencies. Time constant R_1C is adjusted to the same value as that produced by stray feedback capacitance in the I/V amplifier; R2 provides damping to prevent oscillation. If the patch clamp input stage amplifier has a more complicated response, as is the case with commercial switchable resistor designs, then additional waveform shaping circuits are used to compensate the response.

Setting up the compensation: this is most easily done with a square wave current injected into the input, adjusting R, (either a panel mounted or internal potentiometer) to give a square output, flat topped up to ~10 ms. A square input current is achieved by capacitively coupling a good triangular voltage waveform to the input, simply by clamping the open end of a screened cable in the vicinity of the input pin. The more complicated commercial patch clamps may require adjustments of 4 or more trimpots. The response should be checked with both high amplitude signals (often

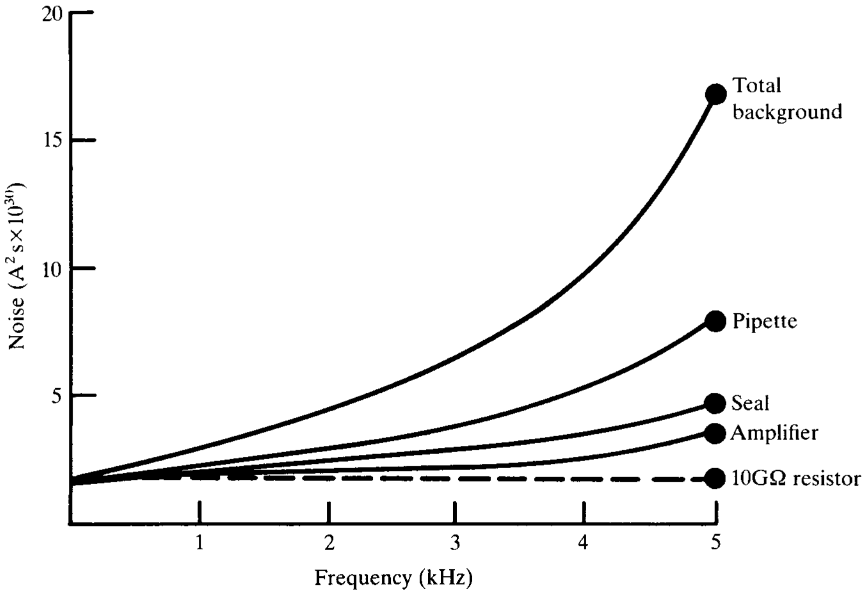
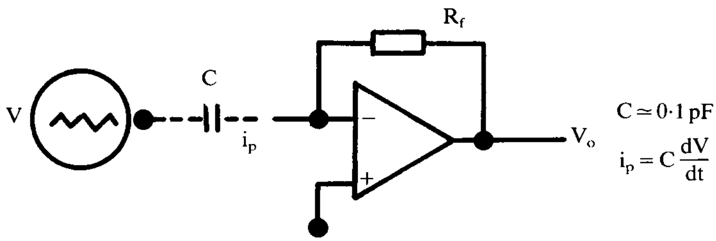
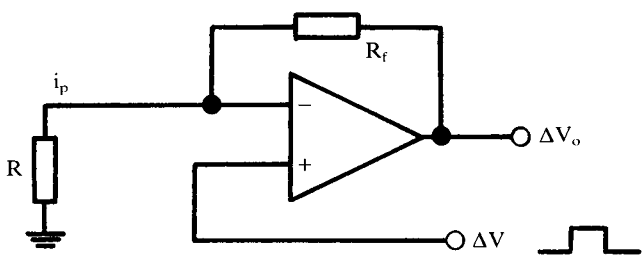


Fig. 9. Contribution of noise from different sources. From Sigworth (1983) with permission.

provided in commercial amplifiers) and low (<10 pA) amplitude as encountered in single channel recording.



Setting the gain: this is done by connecting a precisely know resistance (about 100 MΩ) between input and ground, applying a voltage step V to the non-inverting (V_{ref}) or command input to give i_p = V/R and looking at the output deflection V_o:

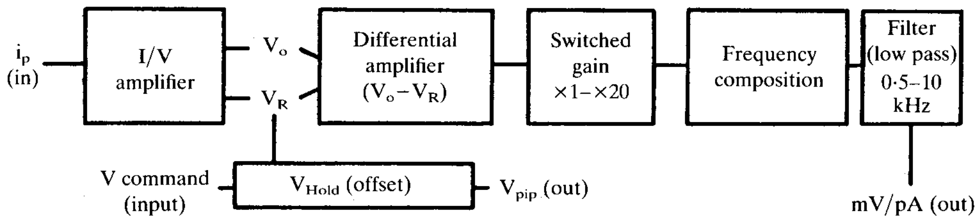


Thus the gain (V/A) is

$$\Delta V_O/i_P = \frac{\Delta V_O R}{\Delta V}$$

The gain is adjusted to give convenient units of V_O/i_P e.g. 10 mV/pA by an internal potentiometer at a later stage of amplification.

The current measurement circuitry of a patch clamp usually consists of the following stages:



It should be noted that the polarity of the output of the initial I/V amplifier stage is retained (i.e. not inverted at a later stage) in most commercial instruments (one exception is the Biologic™), so $V_O = -i_P R_f$.Gain, with i_P positive for current into the amplifier input. Outward membrane currents are negative pipette current in whole cell clamp and outside out patch, and V_O changes in accord with the convention that outward movement of cations is positive. For cell attached recording and inside out patch inward current would deflect V_O positive. Data should be inverted when necessary to conform to the convention.

Capacitor feedback in patch clamp amplifiers

The noise due to the high value feedback resistors used in most patch clamp amplifiers can be avoided by using a capacitor as the feedback element, resulting in an output that is the integral of the pipette current and which is differentiated at the next stage of processing. As well as the absence of Johnson noise, capacitor feedback gives a wider range of current input, which is restricted by the rate of change of the amplifier output rather than the amplitude of the voltage applied across the feedback element. The I/V amplifier is in this case an integrator and suffers the restriction, discussed earlier, that standing offset currents (e.g. through the seal) are integrated along with the signal. The feedback capacitor therefore requires discharge as the potential across it approaches the maximum that can be supplied. This is done by automatic switching that result in reset transients of ≈50 µs in the record. The real advantage for patch clamp recording is an ≈30% reduction in noise that can be achieved with careful single channel recording.

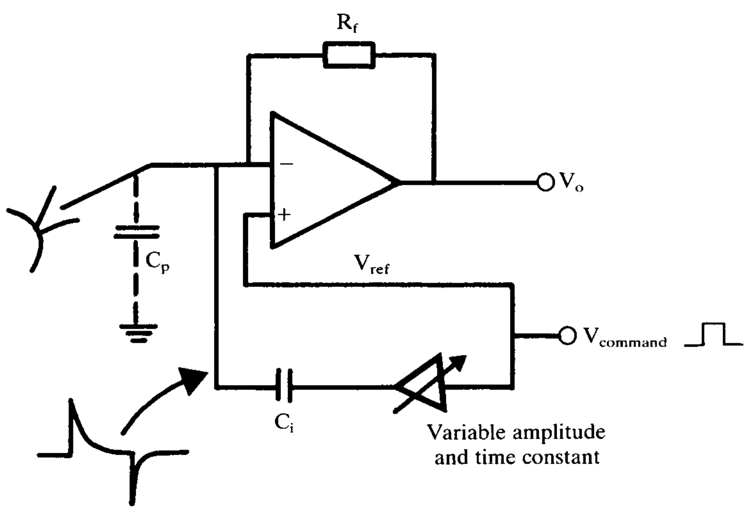
The wide range also has advantages in recording large synaptic currents and in lipid bilayer recording. The properties of the capacitative feedback amplifier are discussed by Finkel (1991).

Voltage command inputs

High resistance seals permit large changes of potential in the pipette without large current flow across the seal into the pipette from the bath. In response to a voltage step applied to V_{ref}, the I/V amplifier produces an output such that current flow through R_f causes the same potential change in the pipette. In order to improve the signal to noise, so as to reduce the contribution of noise on the command, these are divided by a factor, often 10, in the I/V amplifier i.e. V_{ref}=0.1 $V_{Command}$.

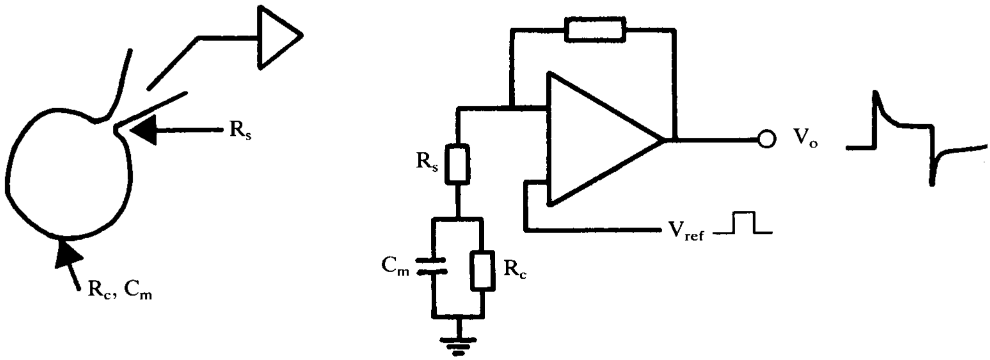
The current flowing into the pipette is the sum of currents (a) through the membrane patch (e.g. single channel currents), (b) through the seal resistance and (c) to charge the input capacity of the amplifier and pipette; (a) is the quantity measured, (b) produces a small, relatively constant offset with good seals, (c) produces large transients in response to rapid potential changes (i=CdV/dt) and may lead to saturation of the I/V amplifier or frequency compensation circuit. These transients obscure single channel currents following a voltage step, and if saturation of the amplifier occurs, may result in loss of voltage control in the pipette and, because of the long recovery times of amplifiers in the circuit, loss of data for several ms following a step.

To make recordings of voltage gated channel currents it is essential to compensate for the transient initial capacity current. Compensation is applied to the pipette input of the I/V amplifier *via* a capacitor (C_i, value ≈2 pF) so as to supply capacity current that would otherwise be provided by the output of the I/V amplifier. For this purpose, the voltage command applied to V_{ref} is modified by a separate parallel circuit, so that its amplitude and risetime can be varied, inverted and injected via the capacitor into the input. It is adjusted to remove the capacity transients to give a square, resistive initial step on the 'pipette current' output.



C_p=stray pipette capacity, C_i is the capacitor for injection of compensation current.

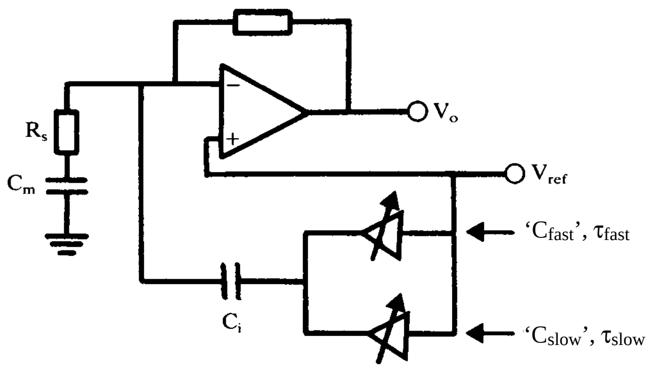
Whole cell recording. The recording of currents from whole cells, after breaking the membrane between pipette and cell, has electrical problems associated with the extra cell capacity to be charged through the series resistance of the pipette/cell junction:



Voltage pulses to V_{ref} (and hence the pipette) cause current to flow into the cell through R_s to charge the cell capacitance, C_m. This gives rise to a current output with initial transients of time constant

$$\tau_c = \frac{R_s R_c C_m}{R_s + R_c} \quad \text{(N.B. for } R_c \gg R_s, \tau \simeq R_s C_m\text{)}$$

and an initial amplitude V/R_s. Thus, after compensation for fast transients, these much slower transients may be used to calculate R_s and C_m. The capacity current required to charge C_m may be compensated in the same way as for the pipette capacitance, i.e. by applying a current of variable magnitude and risetime to the input by injection through the capacitor C_i. This slow capacitance compensation is generated in parallel with the fast compensation and added to the signal injected through C_i. Calibration of the compensation circuit allows C_m and R_s to be read from dials on front panel calibrated potentiometers.



Unlike a microelectrode amplifier, the use of slow capacitance compensation does not improve the speed of clamping the cell membrane potential (unless the amplifiers have reached saturation) but simply removes the capacitative transient from the pipette current and amplifier output. The limiting frequency characteristics of the response of the cell for noise analysis or to a potential step has $\tau \approx R_s C_m$ as before.

The presence of the series resistance R_s gives rise to an error voltage between the clamped pipette potential and the true value of the cell membrane potential,

$$V_c - V_p = i_p R_s.$$

Thus, it is important to know the value of R_s so that corrections can be applied to current/voltage data for the error of V_c. Also, as mentioned above, the transient response of the system is attenuated by the $R_s C_m$ time constant, which for cells of e.g. 10-20 pF and R_s of 10-20 MΩ may produce low pass filtering at around 400 Hz.

The effect of R_s is to underestimate V_c by an amount proportional to the recorded current. Compensation for this effect ('series resistance compensation') may be made by feeding back a proportion of the current signal to V_{ref}. In commercial amplifiers the value of series resistance is taken from the whole cell transient cancellation, multiplied by i_p and a proportion, up to about 80%, and added to V_{ref}. However, this often results in instability and some adjustment to the phase of the compensation may be present. In practice the value of R_s often increases or fluctuates on a minute or shorter timescale during recording, often making series resistance compensation imprecise. As with other cases where compensation is applied, the best results are obtained with a low initial series resistance.

8. Grounding and screening

Ground lines or earths serve four distinct functions in electrophysiological equipment. These are:

(1) *Safety*. All instrument cases and other enclosures of apparatus with a mains supply must have a reliable connection to the mains earth through the plug.

(2) *Reference potential*. Provide the reference (zero) potential for measurements of cell potentials and also for each stage of signal processing that occurs within instruments. Any unwanted signal present on the reference ground of an amplifier will appear in the output and be passed on to the next stage. The reference point is the signal ground of the input amplifier or oscilloscope input. Leads connecting the signal ground should carry no current and run next to or twisted with the signal from the input amplifier, to reduce the area of loop susceptible to magnetic interference.

(3) *Current returns.* To return current to the common of power supplies from e.g. zener diodes, relays, lamps, decoupling circuits. These common returns should be run separately from reference grounds to a central grounding point.

(4) *Screening.* Electrostatic screening to prevent interference from mainly 50 (or 60) Hz is achieved with a Faraday cage and by ensuring that all conducting mechanical parts such as microscope objective, condenser and stage, the baseplate and micromanipulators have good, low resistance («1 Ω) connections to ground. The closer a component is to the pipette, the more important a good connection.

Magnetic interference from transformers or motors occurs by induction in circuit loops of large area oriented across the magnetic field generated. This form of interference is often of 100 or 150 Hz and can be prevented by re-routing ground lines to minimize the area presented to the field, or by moving the source of the interference.

It is usual to arrange grounding to a central point, often on the oscilloscope input, running separate lines for reference and screens to equipment in the cage or rack. Current returns go to the common of the power supply first, which is in turn connected by a single wire to the central ground point. The central point can be taken to the mains earth *via* the oscilloscope cable or by a separate lead.

9. Test equipment

Testing can mostly be done with electrophysiological apparatus already present i.e. an oscilloscope, a precisely timed square wave or pulse generator, such as a Digitimer, and an accurate voltage source or calibrator. Digital multimeters (or DMM) provide accurate measurement of steady potential, current and resistance. A good signal generator or other equipment for occasional use can often be shared or borrowed.

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