experimental arrangement this is of no consequence, but if, for example, a second recording microelectrode is introduced, the changing reference potential produces complications. Again, the changing reference potential is likely to interfere with the correct operation of current sources used for ionophoresis. The subtractive method is free from these difficulties since the reference potential is held constant, usually at 0 V.

The third reason is more subtle. In Fig. 36(a) the three components of the total input capacitance (\(C_i, C_e, C_s\); see Chapter 3, Section V and Chapter 4, Section II.C) are in parallel as seen by the preamplifier and current source. Adjustment of the negative capacitive circuitry \(C_i\) and \(A\) effectively removes them from consideration, and all is well. In Fig. 36(b), however, the transmural capacitance \(C_i\) of the microelectrode bears a different relation to the REF OUT signal than do the other components \(C_e\) and \(C_s\); REF OUT sees \(C_i\) in series and \(C_e\) and \(C_s\) in shunt. This asymmetry is closely related to that discussed in Chapter 4, Section II.D. Analysis shows that with full capacitance compensation applied the time derivative of REF OUT appears at the preamplifier input. Since the derivative of a step voltage change is an “impulse” of infinite amplitude, this means that the preamplifier’s dynamic range will be exceeded by a spike at the start and end of a current pulse. The amplifier will remain saturated for a time depending on its overload characteristics but roughly proportional to the product \(R_{se}C_i\). The resulting loss of information can be severe, especially since one of the best methods for adjustment of bridge balance (Section E below) needs an accurate portrayal of events occurring in the first millisecond or so after a pulse is applied.

E. How to Balance the Bridge

Decide whether accurate bridge balance is needed. If you only want to measure spike frequency of a neurone during current passage, it is best to leave the bridge frankly unbalanced, adjusting the BAL control merely to prevent the trace from disappearing off the oscilloscope screen.

Accurate balance is possible only if the microelectrode resistance is constant. Real microelectrodes are non-linear during current passage (Chapter 3, Section VII). Test the electrode before impalement to find the extent of its quasilinear region, remembering that some electrodes behave better for outward current pulses and others behave better for inward pulses. The all too common behaviour of Fig. 38(a) and (b) excludes an electrode from accurate experimentation.

A primitive method is to balance the bridge with the microelectrode

extracellular, then to impale and hope that \(R_{se}\) has not changed. This method nearly always gives the wrong answer, often grossly so (Schanne, Kawata, Schäfer and Lavallée, 1966; Schanne, 1969). The resistance may decrease during impalement owing to partial breakage of the tip. Usually, though, it increases because the conductivity of intracellular fluid is less than that of extracellular fluid.

The input resistance of most cells with action potentials falls to a low value at the peak of the spike; at this time the change in potential seen as a result of current injection is almost entirely due to \(IR_{se}\). Bridge balance can therefore be adjusted so that injection of current does not alter the displayed overshoot (Martin and Pilar, 1963). The method fails if the cell does not have a suitable action potential, or if for some other reason an action potential cannot be evoked. Severe inaccuracies result if the injection of current alters the true overshoot.

The best method is that popularized by Engel, Barclon and Eisenberg (1972). In essence, the idea is to adjust the balance so that the displayed trace “looks right”, i.e. so that the charging of the membrane capacitance at the onset of the pulse begins smoothly from the baseline potential. The unwanted component \(IR_{se}\) of the total potential is identifiable by its abrupt onset coincident with the applied current, whereas the wanted signal lags in time owing to the membrane capacitance. The method would be completely obvious and simple were it not for the stray capacitance of the input circuit. The effect of this is to distort the wanted signal and, much more importantly, to cause a lag in the time course of the injected current \(I\). The latter effect results in a
disparity between the voltage $IR_{m}$ and the balance signal; a transient artefact appears on the oscilloscope screen (Fig. 39(a)). Its time course is governed by the time constant $R_{m}C_{m}$. To allow accurate bridge balance this time constant must be much shorter than the cell's charging time. Careful adjustment of negative capacitance is nearly always needed to ensure that this condition is met (Fig. 39(b), (c)). The adjustment must be made while the responses are viewed at high sweep, say 0.5–5 ms per division. When full capacitance compensation has been applied the transient artefacts at the start and end of the current pulse are reduced to vertical spikes (Fig. 40). Any low-pass filter in the signal chain should be switched off to avoid the effect of Fig. 30(b).

**F. Errors and Artefacts**

Most errors result from faulty bridge balance or from attempts to pass too much current through high resistance microelectrodes. An hour's practice with the analogue circuits of Fig. 41 will reveal any limitations of instrumentation or bridge-balancing technique. There is no simple way

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**Fig. 39.** Adjustment of bridge balance during injection of current. (a) No negative capacitance applied. (b) Correct application of negative capacitance reveals faulty bridge balance. Note the abrupt steps preceding the take-off points (arrowed) for the charging of membrane capacitance. (c) The bridge has been accurately balanced. (d) Too much negative capacitance.

**Fig. 40.** A moderately successful example of current injection. The responses were obtained from a sympathetic neurone of a rat embryo, grown in cell culture for 4 days. Note the spiky transient at the start and end of the current pulses. A small degree of bridge imbalance is evident.

**Fig. 41.** Suggested test circuits for practising bridge balance, in increasing order of difficulty from left to right. The parallel combination of $R$ and $C$ (10 MΩ and 1 nF in each case) represents the passive electrical properties of an impaled cell.
of overcoming the limitations of microelectrodes, although bevelling (Chapter 2, Section VI) should help.

Current injected from a microelectrode has to traverse the cytoplasm as well as the cell membrane, and in so doing it produces potential gradients within the cell. The transmembrane potential changes evoked by current flow cannot therefore be the same at all parts of the membrane, except in the very special case of the microelectrode's being at the centre of a spherical cell. The consequent difficulties of interpretation, some of which are fortunately more hypothetical than real, have been discussed by Engel et al. (1972), Peskoff and Eisenberg (1975) and Purves (1976). These comments apply equally to the single-microelectrode and two-microelectrode methods of current injection with simultaneous potential measurement, although the precise effect of cytoplasmic current flow differs in the two methods.

The transient artefacts (Fig. 40) discussed in the previous section are an important guide to the adequacy of capacitance compensation and bridge balance. At one time it was fashionable to introduce extra circuitry to suppress the artefacts, with the inevitable result that the balancing method of Engel et al. (1972) could not be properly executed. This may be why many people regard the technique of current injection with suspicion. One often reads a ritual disclaimer to the effect that such

and such a result must be taken as approximate because it was obtained by a single-microelectrode method. A statement like this conceals one of two quite different circumstances. The experimenter may simply not have known how to adjust bridge balance; in this case the result is not "approximate" — it is wrong. Alternatively, it may genuinely have been difficult to locate the correct bridge balance; this occurs when high resistance microelectrodes are used on cells whose charging time constant is short. In favourable circumstances, as when the impaled cell has a high input resistance and long time constant, the single-microelectrode method need not be used apologetically for it is capable of high accuracy.

It will be recollected that a small part of the transmural capacitance C, of a microelectrode is "distributed", i.e. mixed up with the resistance of the tip region (Chapter 3, Section V). Microelectrodes of high resistance, immersed deeply within the bathing solution, may show transient artefacts that cannot be converted into vertical spikes by application of full capacitance compensation (Fig. 42). The resulting inaccuracies of bridge balance are usually rather small; the only practicable way to eliminate them is to reduce the depth of the bathing medium.

G. Multiplex Method

Some of the difficulties associated with bridge balance and electrode non-linearity can be overcome by applying the injection current as a series of pulses, the membrane potential being measured only during the intervals between pulses. Electronic switching can be arranged to allow very rapid alternation between current injection and voltage measurement. If the measurement phase is sufficiently short with respect to the cell's input time constant, the membrane potential will not "droop" significantly, and the effect is one of continuous current injection and potential measurement. Bader, MacLeish and Schwartz (1979) and Merickel (1980) give circuits and discuss voltage-clamping with a single microelectrode (Wilson and Goldner, 1975). The multiplex method requires the time constant of the input circuit to be extremely small; the preamplifier must have well-designed negative capacitance circuitry.