CHAPTER 18

Determination of Neuronal Membrane Properties
Using Intracellular Staining Techniques

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As previous chapters in this book demonstrate, recently developed Procion dye injection techniques make it possible to study both the electrophysiological and the morphological properties of single neurons. This chapter describes how the electrophysiological and anatomical information derived from the use of dye-filled pipettes can be used to calculate passive membrane properties of nerve cells. Knowledge of the membrane parameters enables prediction of the size and relative effectiveness of synaptic potentials originating on the dendritic tree (Rall, 1962, 1967, 1970). In order to obtain accurate data, particular attention must be paid to micropipette recording techniques, and these will be discussed in the first part of this chapter. The experiments reported here were performed on cat motoneurons, but the techniques should be applicable to many other cells.

Intracellular Measurement of Neuronal Input Resistance

Input resistance is measured by recording the steady-state voltage response of the neuron after a small step of current is applied through an intracellular electrode. Ideally, the voltage-recording and current-passing electrodes should be completely separate, with no resistive or capacitative coupling. It is very difficult, however, to record healthy responses from motoneurons impaled by two separate electrodes; therefore, most studies of input resistance employ single- or double-barreled micropipettes.

Single-barreled Electrodes

The easiest method of measuring input resistance is to use a single barrel both to record voltage and to pass current. This technique minimizes damage to the neuronal membrane. However, when voltage is recorded from the same electrode that applies current, a bridge circuit must be used to subtract the current-induced voltage drop across the electrode tip. The bridge can be balanced before penetrating the neuron or, after penetration, by assuming that the very fast components of the voltage response

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to a current step originate across the electrode tip, and that only the slower components originate across the neuronal membrane (Nelson & Frank, 1967).

Unfortunately, the high and often variable resistance of dye-filled pipettes makes it difficult to balance the bridge accurately. Errors in bridge balance can be reduced by (1) lowering the resistance of the electrode, by mixing the dye solution in 0.15 M KCl and by beveling the electrode tips (see section on Electrode Beveling Techniques) and (2) by checking bridge balance when the resistance of the neuronal membrane is shunted by the conductance changes accompanying an intense synaptic barrage (e.g., stimulation of the dorsal roots at 1000 Hz for 50 msec). The bridge balance achieved with such stimulation usually agrees well with the balance obtained by separating the fast and slow components of the voltage response, as described above, but both methods could overestimate the balance slightly and so underestimate neuronal input resistance.

The damage to motoneurons caused by the enlarged tip openings of the beveled pipettes does not appear to be greater than that produced by standard, nonbeveled tips. Resting potentials, action potentials, and time constants of motoneurons impaled with beveled, dye-filled pipettes were similar to those recorded with standard fine pipettes (Barrett & Graubard, 1970). Beveling seems to facilitate cell penetration, and the lower resistance of the beveled pipettes permits more accurate recording of transient voltage responses. When beveled electrodes are filled with standard 3 M KCl or K-acetate solutions, however, diffusion of these extremely hypertonic solutions from the enlarged tip may damage neurons.

**Double-barreled Concentric Electrodes**

When a motoneuron is impaled with both barrels of a concentric electrode, and the inner electrode tip then advanced 5 to 10 μm ahead of the outer barrel, the resistive (or DC) coupling between the barrels can be reduced well below the neuronal input resistance (Tomita, 1969). Such decoupling allows very accurate measurement of the steady-state input resistance of neurons. Concentric electrodes offer no advantage in measuring transient voltage responses because of the large capacitative coupling between the inner and outer barrels. The distributed nature of this coupling appears to make it impossible to balance the capacitative artifact completely with a cross-compensation circuit.

**Double-barreled Electrodes drawn from θ-tubing**

Intracellular recording from motoneurons is relatively easy with double-barreled electrodes made from θ-shaped tubing, but the neighboring barrels share a common resistive pathway to ground (Coombs et al., 1955; Nelson and Frank, 1967). Grinding the tip of the θ-tubing electrode at an angle perpendicular to the center partition reduces this shared resistance, and also reduces the resistance of each barrel. The remaining 0.1–0.5 MΩ coupling resistance must be balanced by a bridge circuit. Capacitative coupling between the barrels is less than in concentric electrodes, but the capacitative transient still distorts the first 0.1–0.2 msec of the voltage response to a current step, and thus makes accurate bridge balance difficult. The capacitative coupling between both concentric and θ-tubing barrels is somewhat reduced when only the first millimeter of the electrode tips is filled with electrolyte.