Chapter 9
The Cut-Open Axon Technique

FRANCISCO BEZANILLA and CAROL VANDENBERG

1. Introduction

The giant axon of the squid has been the preparation of choice for the recording of electrical events associated with the opening and closing of the ionic conductances. Its large size allows the introduction of electrodes and the exchange of solutions giving almost complete control of the chemical environment and voltage across the axolemma (Adelman and Gilbert, 1990; Mullins and Brinley, 1990).

The giant axon has provided a wealth of information on the macroscopic ionic currents and gating currents, two of the three electrical expressions of voltage dependent ionic conductances. The third expression, single channel fluctuations, is normally done with the patch clamp technique that requires a clean glass pipette pressed against a clean membrane. This technique has been difficult to apply to the giant axon due to the presence of external connective tissue and the Schwann cell layer. Conti and Neher (1980) introduced a bent pipette inside a perfused axon to record the fluctuations of single potassium currents and this work was the first direct demonstration that the squid potassium current is produced by a large number of discrete channels. However, the technique had a limited frequency response and it was not possible to achieve gigaseals due to the presence of remaining axoplasm. A similar technique was used by Lopez-Barneo et al. (1981) to record from a small population of channels in the giant axon.

2. The cut-open axon technique for small population of channels

A different approach was to cut the axon open to have free access to the internal surface of the membrane. The original technique (Llano and Bezanilla, 1980) consisted of isolating a small segment of axon and cutting it open with microscissors over a hole of about 200 μm on a plastic partition separating two compartments. This operation exposed the internal surface which became oriented upwards. The solutions in both compartments could be exchanged and by introducing electrodes it was possible to...
voltage clamp the axon segment using a conventional negative feedback voltage clamp circuitry. The seal between the axon and the plastic partition did not exceed 1 Mohm which constituted a short circuit for the normal conductances, eliminating the resting potential and making the distribution of the potential non-uniform in the segment. Even though macroscopic and gating current could be recorded with this technique, the main objective was to isolate small patches of membrane using patch clamp pipettes. The frequency of gigaseals was very low and only a few successful recordings of potassium channels were obtained (Llano and Bezanilla, 1983). Most frequently, seals of tens of megohms were obtained and fluctuation studies of small population of channels were undertaken (Llano and Bezanilla, 1984) using the technique introduced by Sigworth (1980).

Another cut-open axon technique was introduced by Bekkers et al. (1986) and described in detail by Forster and Greeff (1988). In their technique the axonal membrane was sandwiched between the two apices of cones machined in plexiglass. This method allowed recording of fluctuations of a large number of channels (ca. 10⁶), but was not intended to be used with patch pipettes.

3. The cut-open axon technique for single channel recording

The original cut-open axon technique demonstrated that it was possible to slit the axon open and still have functional channels. During the procedure of cutting the axon open, care was taken not to expose the internal side of the membrane to external solutions such as sea water. It was found later by Levis et al. (1984) that actually, the exposure of the internal face of the axonal membrane to sea water did not eliminate all the functional channels and made possible the recording of several thousand to millions of channels with large glass pipettes pressed against an open piece of axon.

This technique gave origin to a method to record single channels from the cut-open axon (Llano and Bezanilla, 1985). The exposure to sea water was beneficial in obtaining gigaohm seals with excellent reproducibility by activating proteases present in the axoplasm which helped in cleaning the internal surface of the axon membrane. This modified cut-open axon technique was first used to record potassium channels (Llano and Bezanilla, 1985; Llano et al., 1988) and then single sodium channels (Bezanilla, 1987), macroscopic currents, and even gating currents with patch pipettes (Vandenberg and Bezanilla, 1988). A similar method has also been applied for the recording of single sodium channels from the cut-open Myxicola giant axon (Schauf, 1987).

4. Method and results

4.1. Experimental set-up

The set-up is built around a modified inverted microscope. Some of the modifications are standard for patch clamp recording, such as a movable stage independent from the microscope body and a strong base for the motor driven micromanipulator used to approach the patch pipette. The stage is modified to set an