Book Reviews

The Patch Clamp

Single-Channel Recording. BERT SAKMANN and ERWIN NEHER, Eds. Plenum, New York, 1983. xxii, 503 pp., illus. $50. From a course, Erice, Sicily, summer 1982.

Following the conception of the voltage clamp by Cole and its exploitation by Hodgkin and Huxley, the technique was improved over several years and gradually became adapted to use on a variety of cells other than the original squid axon. In contrast, following the observation published in 1980 by Sigworth and Neher that high-resistance (gigohm) seals ("gigaseals") could be obtained between fire-polished microtips and cell membranes, the development of the patch clamp and of applications of it has proceeded at an astonishing—if not explosive—pace. In 1981 Hamill, Marty, Neher, Sakmann, and Sigworth published a landmark paper discussing the variety of applications of this technique, which allows not only observations of membrane ionic currents from a whole cell but also ion flow through single voltage-sensitive channels in a membrane patch of a few microns.

This is spectacular sensitivity: changes in the configuration of a single molecule can be inferred from the opening and closing of a single channel. In addition to the expected distribution of ion channels' openings and closings, there are now a wealth of observations of extra periodicities and some of multiple levels of open conductance.

An unexpected dividend of this methodology is the ability to follow changes in membrane capacitance, apparently caused by exocytosis and endocytosis associated with neurosecretion or transmitter release (for example, the fusion of synaptic vesicles with the cell membrane followed by retraction of bits of membrane within the cell).

The focus of this work has been at the Max-Planck-Institut für biophysikalische Chemie in Göttingen in the laboratories of the coeditors, but personnel (past and present) in the laboratories of C. F. Stevens (Yale Medical School) have also been major contributors.

At a course on the patch-clamp technique that brought together people from most of the laboratories involved in patch clamping at the time it became apparent that the technique had reached a state of maturity, and it was decided to produce this volume, which gives detailed descriptions of all the aspects of the patch-clamp technique, including representative examples of results.

The first section of the book, Methods, begins with a chapter on the electronic design of the patch-clamp circuitry by Sigworth, who sets out the problems and solutions. The engaging and lucid style of this chapter is maintained throughout the book. Sakmann and Neher provide extensive details on the geometric parameters of pipettes and membrane patches, giving the characteristics of soft and hard glass. Corey and Stevens discuss possible mechanisms for the seal between glass and membrane and give extensive detail on the choice of glass and the pulling, coating, polishing, and filling of pipettes. There are discussions of methods for the dispersion of cells (Trube) and a primer on cell culture (Spector). Tank and Miller describe patch-clamped liposomes and the recording of reconstituted ion channels. A chapter on tight-seal whole-cell recording (Marty and Neher) is followed by one on a more restricted "loose patch clamp" method (Stuhmer, Roberts, and Almers).

In the second section, Concepts and Analysis, Colquhoun and Hawkes develop a most illuminating stochastic interpretation of ion-channel mechanisms. They relate reaction mechanisms and rate constants to the probabilities of channel openings and closings and address the question of the burst of ion-channel openings. Läuger discusses the relation of the single-channel data to changes in molecular conformation.

Colquhoun and Sigworth provide an excellent treatment of methods of acquisition, filtering, event detection, statistical analysis, and fitting of distributions (and include computer programs). Aldrich and Yellen give an example of the use of conditional probability (available only in single-channel data) to test kinetic models in a way not possible with only macroscopic data. DeFelice and Clay relate single-channel kinetics to the probabilistic interpretation of Hodgkin-Huxley kinetics.

Examples of single-channel data from a variety of preparations are presented in the final section, Patch Clamp Data. Acetylcholine-receptor channels show bursts of openings (Colquhoun and Sakmann) and multiple conducting states (Sachs). There are two chapters on analysis of data from glutamate receptors, one by Gration, Ramsey, and Usherwood and the other by Cull-Candy and Parker. There are also chapters on cholinergic chloride channels (Ascher and Erulkar), serotonin-modulated potassium channels (Camardo and Siegelbaum), calcium channels (Lux), and channels in red blood cells (Hamill).

Perhaps the only thing missing from this splendid volume is a statement of the limitations of and problems with the method. The patch clamp can be used only on cells with relatively clean surfaces (uncomplicated with wrappings of Schwann cells, for example). This makes it much more practical for dissociated or cultured cells than for recording in situ. Furthermore, once a pipette tip has touched a cell without making a seal, its surface is contaminated so that it is unable to make one thereafter.

Although the data from single channels contain information on the opening and closing of channels that is missing from macroscopic records, it is still insufficient for choosing among a variety of possible kinetic models for the channel gating mechanism. Horn and Lange (*Biophysical Journal*, August 1983) have developed a powerful (and computation-intensive) method for estimating rate constants for a given kinetic reaction from single-channel data. They conclude that selecting a reasonable kinetic model requires a knowledge of the number of states, the way they are connected, which ones are open or closed, and the probability of a given channel's being in each of the possible kinetic states. Horn and Lange conclude that "selecting the correct model is largely a matter of experimental intuition and the availability of other information, such as detailed macroscopic data, noise measurements, histograms of single channel currents and gating currents."

Though one of the early and worthy goals of patch clampers observing single-channel kinetics was to relate these kinetics to macroscopic observations, it turns out that that has not been easy for several reasons: (i) In excised patches, the voltage sensitivity of the single channels is often tens of millivolts displaced from the macroscopic observations. On the other hand, with cell-attached patches the membrane potential is unknown. (ii) The time required to gather sufficient records to establish voltage sensitivities

and temporal kinetics is considerably greater than the time of survival of single channels in patches. (iii) Single-channel records taken with a cell attached may differ drastically from those taken in excised patches.

Although it has been difficult up to now to show much relationship between microscopic and macroscopic currents, the single-channel data open up a whole new array of previously unexpected detail, both in multiple time-constant kinetics with several kinds of bursting and in multivalued channel conductances. It appears that this situation might be analogous to that encountered earlier with atomic spectra (with fine and hyperfine structure), for which the necessary resolution and consolidation were provided by Bohr.

In contrast to the frequently observed lack of coherence in a volume generated from a meeting, this book is a rather tightly integrated mix of "how to" (for example, recipes for making pipettes and cell suspensions), richly detailed descriptions of methods for data analysis (including computer programs for filtering and detection), and scholarly analyses of electronic circuits and the underlying theoretical basis for the interpretation of the channel kinetics. The 1981 article by Hamill *et al.* is reprinted in an appendix.

The book should remain a landmark in the field of membrane biophysics. I appraise it as a "must" book for anyone who is now using or intends to begin research employing patch clamps.

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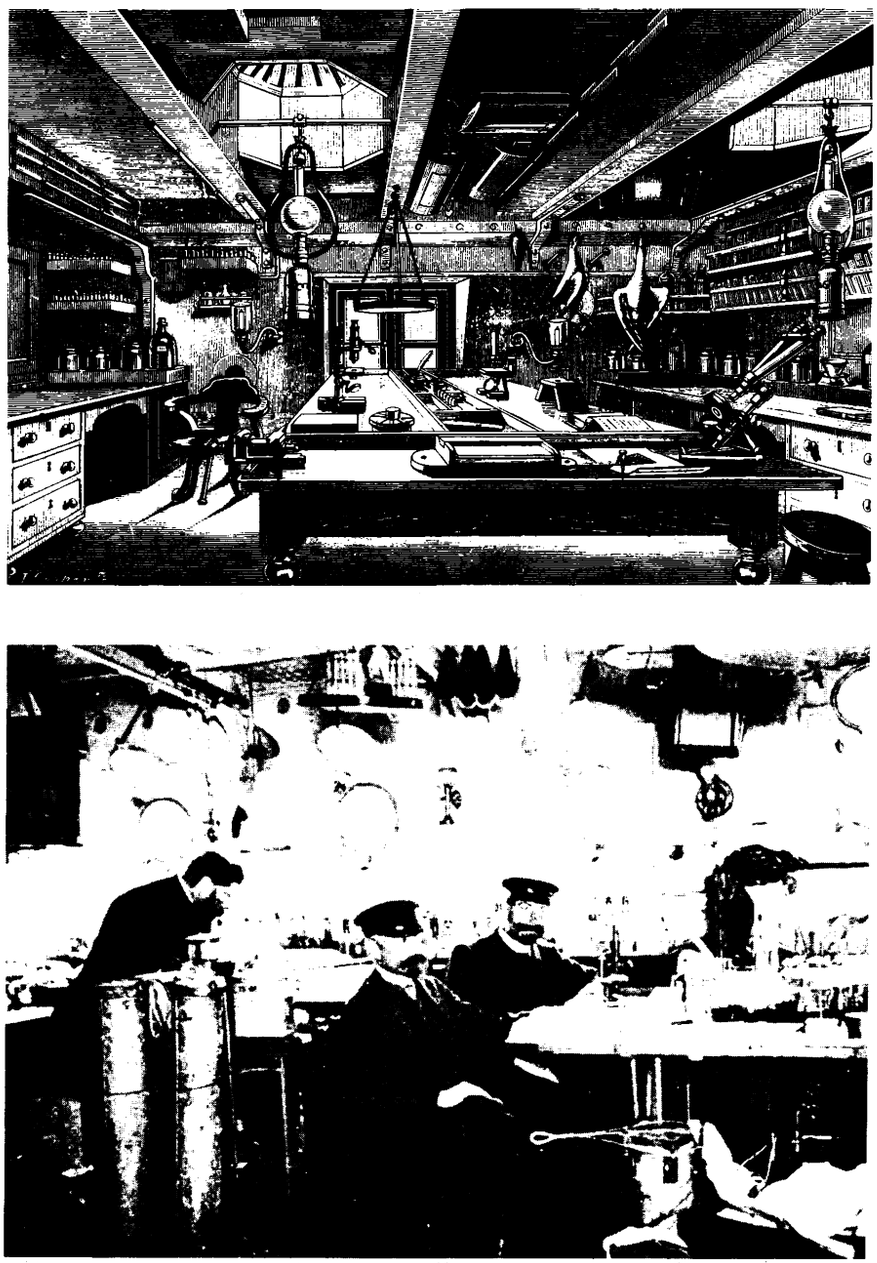
The Benthic Fauna

Deep-Sea Biology. GILBERT T. ROWE, Ed. Wiley-Interscience, New York, 1983. xii, 560 pp., illus. $69.95. The Sea, vol. 8.

Deep-Sea Biology had its genesis in 1977, inspired partly by the discovery of the remarkable communities associated with the Galápagos hydrothermal vents. The editor's aim was to reflect recent progress in this and other broad areas of deep-sea biology, but not to attempt to cover all aspects of the subject. Consequently, acknowledged leaders in selected fields were invited to review their own areas, and in doing so several of them have also tried to identify the most challenging and fruitful topics for future research.

Despite an excellent résumé of the history of deep-sea biology in general by Mills and a chapter by Vinogradov and Tseitlin devoted to mid-water life, the book is essentially concerned with the benthos, that is the organisms living in, on, or very close to the floor of the deep ocean. Three of the four rather arbitrary size categories into which the benthic fauna is usually divided are each dealt with in separate chapters: the bacteria by Jannasch and Wirsen, the nanobenthos and meiobenthos by Thiel, and the macrobenthos by Rowe, in each case with a review of modern techniques and a summary of results. These same size categories are the ultimate subject of a chapter on sediment community metabolism by Smith and Hinga, and the meiofauna and macrofauna provide most of the examples used by Jumars and Eckman to illustrate and summarize current knowl-



"Contrasting styles in sea-going biological laboratories." (Top) "The 'natural history work room' of H.M.S. *Challenger*, 1982 to 1876." (Bottom) "Laboratory of *Princess Alice II* during cruise in summer 1904. The personnel are, from left to right, L. Tinayre (artist), P. Portier (bacteriologist), and Jules Richard (scientific director). During this cruise in the Mediterranean and tropical eastern Atlantic, Portier, using sterilized reversing bottles, showed that the open sea had relatively few bacteria and that none could be grown from sediment samples collected at abyssal depths (a result at variance with earlier studies) although there were always bacteria associated with deep water animals." [From E. Mills's paper in *Deep-Sea Biology*; originals (respectively) from C. W. Thomson, *The Voyage of the Challenger: The Atlantic* (Harper, New York, 1878), courtesy of Institut Océanographique, Monaco)]