Single-Channel Recording

Second Edition

Single-Channel Recording

Second Edition

Edited by

Bert Sakmann
Max-Planck-Institut für medizinische Forschung
Heidelberg, Germany

and

Erwin Neher
Max-Planck-Institut für biophysikalische Chemie
Göttingen, Germany

SPRINGER SCIENCE+BUSINESS MEDIA, LLC

Library of Congress Cataloging-in-Publication Data

Single-channel recording / edited by Bert Sakmann and Erwin Neher. --
2nd ed.
p. cm.
Includes bibliographical references and index.
ISBN 978-1-4419-1230-5 ISBN 978-1-4419-1229-9 (eBook)
DOI 10.1007/978-1-4419-1229-9
1. Ion channels--Research--Methodology. I. Sakmann, Bert, 1942-
II. Neher, Erwin, 1944-
QH603.I54S56 1995
574.87'5--dc20 95-3364
CIP

Cover illustrations by Dr. Claudia Racca (neuron) and Dr. Nelson Spruston (trace).

ISBN 978-1-4419-1230-5

Originally published by Plenum Press, New York in 1995
Softcover reprint of the hardcover 2nd edition 1995

10 9 8 7 6 5 4 3

Contributors

Klaus Benndorf Department of Physiology, University of Cologne, D-50931 Cologne, Germany

Robert H. Chow Department of Membrane Biophysics, Max-Planck-Insitute for Biophysical Chemistry, Am Fassberg, D-37077 Göttingen-Nikolausberg, Germany.

David Colquhoun Department of Pharmacology, University College London, London WC1E 6BT, England

Jens Eilers International Physiological Institute, University of Saarland, D-66421 Homburg/ Saar, Germany

Kevin D. Gillis Department of Membrane Biophysics, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg, D-37077 Göttingen, Germany

Walter Häberle IBM Physics Group Munich, D-80799 Munich, Germany

Owen P. Hamill Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, Texas 77555-0641

Alan G. Hawkes European Business Management School, University of Wales Swansea, Swansea SA2 8PP, Wales.

Rainer Hedrich Institute for Biophysics, University of Hannover, D-30419 Hannover, Germany

Stefan H. Heinemann Max-Planck Society, Research Unit, "Molecular and Cellular Biophysics," D-07747 Jena, Germany

Donald W. Hilgemann Department of Physiology, University of Texas Southwestern, Medical Center at Dallas, Dallas, Texas 75235-9040

J. K. Heinrich Hörber Department of Cell Biophysics, European Molecular Biology Laboratory (EMBL), D-69117 Heidelberg, Germany

Peter Jonas Department of Cell Physiology, Max-Planck-Institute for Medical Research, D-69120 Heidelberg, Germany

Arthur Konnerth First Physiological Institute, University of Saarland, D-66421 Homburg/ Saar, Germany

P. Läuger Department of Biology, University of Konstanz, D-78434, Konstanz, Germany. *Deceased:* direct correspondence to H.-J. Apell at this address.

Alain Marty Neurobiology Laboratory, Teacher's Training College, F-75005 Paris France; *Present address:* Cellular Neurobiology Workgroup, Max-Planck-Insitutute for Biophysical Chemistry Am Fassberg, D-37077 Göttingen, Germany

Don W. McBride, Jr. Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, Texas 77555-0641

Hannah Monyer Center for Molecular Biology (ZMBH), Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany

Johannes Mosbacher Department of Cell Physiology, Max-Planck-Institute for Medical Research, D-69120 Heidelberg, Germany

Erwin Neher Department of Membrane Biophysics, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg, D-37077 Göttingen, Germany

Anant B. Parekh Max-Planck-Institute for Biophysical Chemistry, Am Fassberg, D-37077 Göttingen, Germany

Reinhold Penner Department of Membrane Biophysics, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg, D-37077 Göttingen, Germany

Bert Sakmann Department of Cell Physiology, Max-Planck-Institute for Medical Research, D-69120 Heidelberg, Germany

Ralf Schneggenburger International Physiological Institute, University of Saarland, D-66421 Homburg/Saar, Germany. *Present address:* Neurobiology Laboratory, Teacher's Training College, 46 Rue d'Ulm, F-75005 Paris, France.

F. J. Sigworth Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut 06510

Greg Stuart Department of Cell Physiology, Max-Planck-Institute for Medical Research, D-69120 Heidelberg, Germany

Walter Stühmer Max-Planck-Institute for Experimental Medicine, D-37075 Göttingen, Germany

Ludolf von Rüden Department of Membrane Biophysics, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg, D-37077 Göttingen-Nikolausberg, Germany. *Present address:* Howard Hughes Medical Institute, Department of Cellular Physiology, Stanford University, Stanford, CA 94305-5428

Preface

The single-channel recording technique has reached the status of a routine method, and the view that conductance changes in biological membranes are caused by the openings and closings of ion channels is now almost universally accepted. The most convincing early evidence for channels mediating flow of ions across biological membranes was provided in 1972 by Bernard Katz and Ricardo Miledi through the observation of membrane noise and their estimate of the size of the underlying "elementary event." The patch-clamp method has confirmed their view directly. In 1993, the work of Nigel Unwin permitted a first visual glance through an ion channel in a biological membrane.

In the sense we use it in this book, the concept and the word *Kanal* was used first by the Austrian physiologist Ernst Brücke in 1843 to describe his view of the mechanism of transport of solutes (via water-filled capillary tubes) through a biological membrane separating two fluids. Patch-clamp recording and molecular cloning of channel genes have revealed an enormous diversity of ion channels. As has been found for other proteins, ion channels fall into different families that share common functional properties. Bertil Hille speculated that the very diverse channel subtypes may have evolved from prototypical channels (or even from an "*Ur-Kanal*"). Neither the ion selectivity nor the gating mechanism nor amino acid sequence motifs of the putative channel ancestors are known. Also, the evolutionary relationships between channel families are just emerging. On the other hand, the evolution of the word channel is fairly straightforward to derive (Fig. 1).

The words *channel* (English), *canal* (French), *Kanal* (German), or *canale* (Italian) derive from Latin *canalis* meaning a small water-filled tube or pipe. It is derived from the Greek word καννα, which also means cane, tube, or pipe. Κανα is a loan-word that was adopted by the Greeks from the Semitic word *qanû*, used likewise by the Phoenicians. This root is preserved, for example, in biblical Hebrew as קנה. The word *qanû* is of Assyro-Babylonian origin and means, among other things, a pipe made from reed. The Sumerian equivalent of *qanû* is gi, which designates the common and the giant reed growing in Mesopotamia and in the Near East and which is, in systematic botany, referred to as *Phragmites australis* and *Arundo donax L.* In early Sumerian cuneiform writing the shape of the reed (Fig. 2) is preserved. It is interesting to note that the same word κανα is also the root of such common words as canon or canonical, meaning a set of rules that have reached the status of official truth.

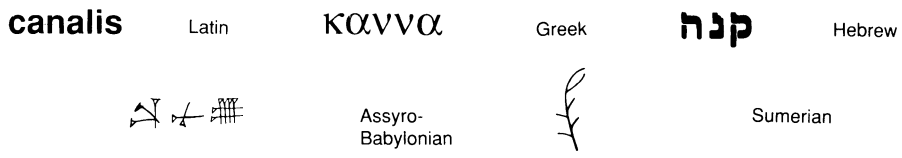


Figure 1. Evolution of the word channel from Sumerogram gi.

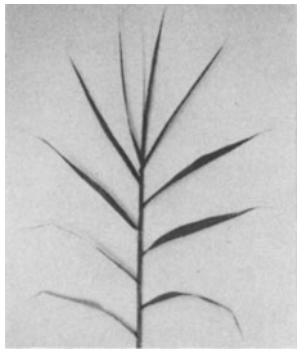


Figure 2. Photograph of giant reed (*Arundo donax* L).

Presumably in ancient times tubes made from this reed were used to suck or expel water and solutes between different reservoirs. Until the present day, *Arundo donax* tubes have been used for making water pipes for houses in the Near East.

Canalis was originally the word for devices used to direct the flow of water and solutes and was only later replaced in western Europe by the French words pipe and pipette. This book is about both channels and pipettes, since the essence of patch clamping is the attempt to join a channel to a pipette in order to measure the flow of solutes through both.

Patch pipettes have become more useful than originally thought; i.e., they are useful not only for measuring flow of ions through channels. In this new edition of *Single-Channel Recording,* we include a number of new chapters that describe techniques that rely on the use of pipettes to study cellular mechanisms that are only indirectly related to single ion channels.

In our opinion some important new applications have developed since the first edition of the "blue book":

- Capacitance measurements allowing the detection of single fusion events of secretory vesicles.
- Single-cell PCR measurements allowing detection of mRNA molecules in single cells by combining patch-clamp methods with molecular biology methods.
- Whole-cell recording from neurons in brain slices in combination with imaging techniques.
- Atomic force microscopy of cells and membranes attached to glass pipettes in the hope of allowing the detection of the structure of molecules in membranes.

The new edition therefore includes new chapters that give accounts of these wider applications. Also, three introductory chapters were added, which are intended to introduce the newcomer to patch clamping and to provide access to the vast literature on patch-clamp technology that has accumulated in the meantime. We do not try to cover all aspects of the technique, since quite recent reviews handling the different areas are available, such as *Methods in Enzymology,* Vol. 207, *The Plymouth Workshop Handbook* (D. C. Ogden, ed., Academic Press) and the *Axon Guide* (distributed by Axon Instruments).

We would like to thank our colleagues who contributed chapters to the new edition and also Prof. Waetzoldt and Dr. Kramer of Heidelberg University and Prof. Dani Dagan from the Technion for their help in tracing the origin of the word channel.

Bert Sakmann
Erwin Neher

Contents

Part I. INTRODUCTION TO PATCH CLAMPING

Chapter 1

A Practical Guide to Patch Clamping

Reinhold Penner

Chapter 2

Tight-Seal Whole-Cell Recording

Alain Marty and Erwin Neher

Chapter 3

Guide to Data Acquisition and Analysis

Stefan H. Heinemann

Part II. METHODS

Chapter 4
Electronic Design of the Patch Clamp

F. J. Sigworth

Chapter 5
Low-Noise Recording

Klaus Benndorf

Chapter 8

Patch-Pipette Recordings from the Soma, Dendrites, and Axon of Neurons in Brain Slices

Bert Sakmann and Greg Stuart

Chapter 9
Patch Clamp and Calcium Imaging in Brain Slices

Chapter 10
Fast Application of Agonists to Isolated Membrane Patches

Chapter 13

The Giant Membrane Patch

Donald W. Hilgemann

Chapter 14

A Fast Pressure-Clamp Technique for Studying Mechanogated Channels

Don W. McBride, Jr., and Owen P. Hamill

Chapter 15

Electrophysiological Recordings from *Xenopus* Oocytes

Walter Stühmer and Anant B. Parekh

Part III. ANALYSIS

Chapter 18
The Principles of the Stochastic Interpretation of Ion-Channel Mechanisms

Chapter 19
Fitting and Statistical Analysis of Single-Channel Records

David Colquhoun and F. J. Sigworth

Chapter 20

A Q-Matrix Cookbook: How to Write Only One Program to Calculate the Single-Channel and Macroscopic Predictions for Any Kinetic Mechanism

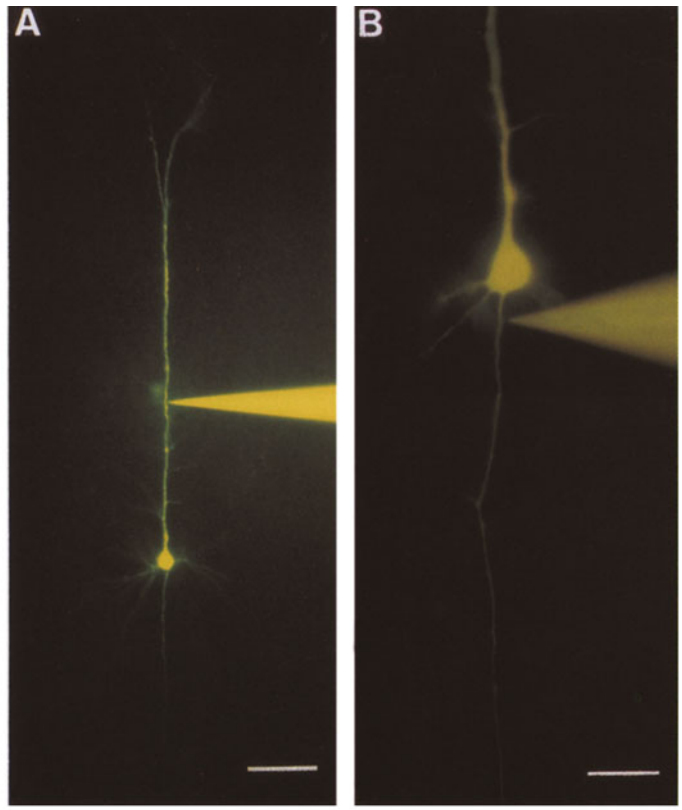
David Colquhoun and Alan G. Hawkes

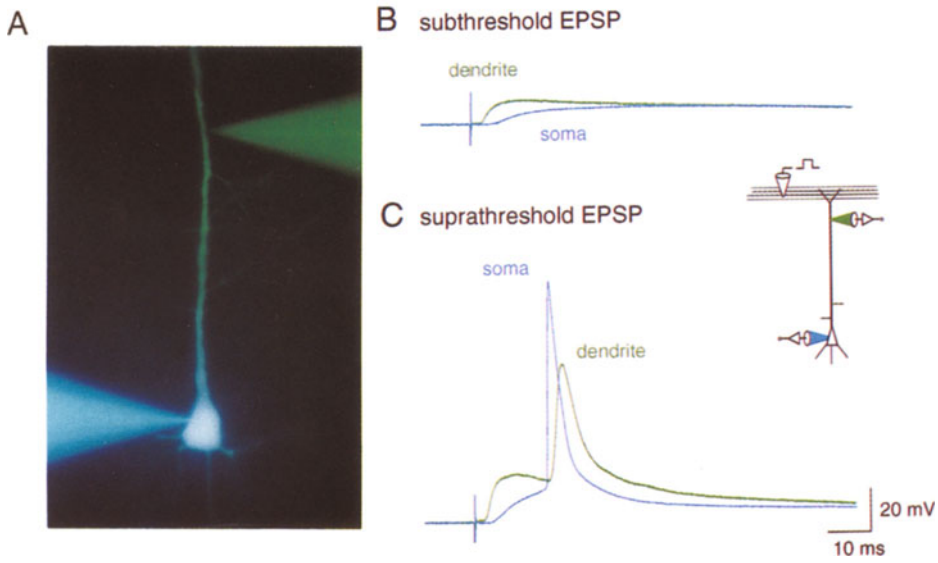
Part IV. "CLASSICS"

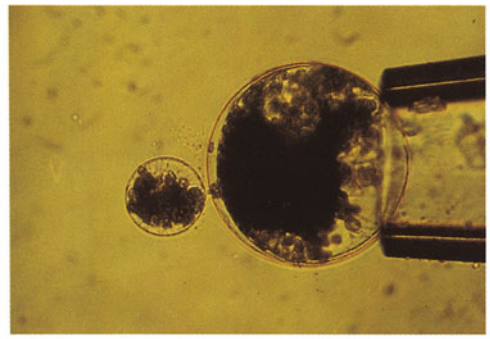
Color Plates

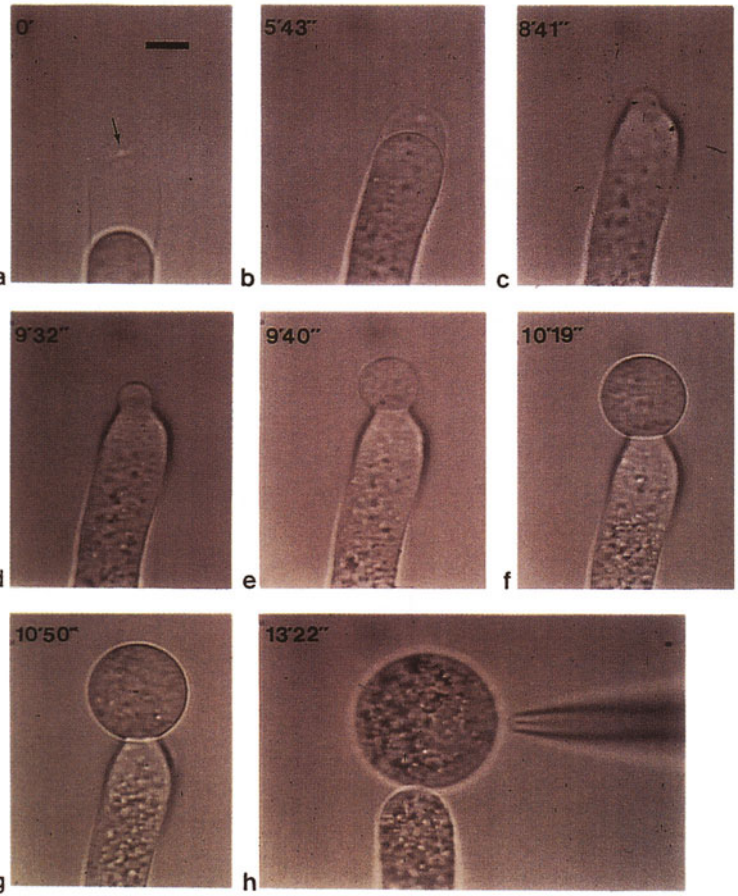
Figure 8-5. Verification of dendritic and axonal recordings by filling of layer V pyramidal neurons with the fluorescent dye Lucifer yellow from the recording pipette. A: Fluorescence photomicrograph of a layer V pyramidal cell following intracellular filling with the fluorescent dye Lucifer yellow via a dendritic pipette located 230 μm from the soma of this neuron. Scale bar 100 μm B: Fluorescence microphotograph of a layer V pyramidal cell following intracellular filling with Lucifer yellow via an axonal pipette located approximately 15 μm from the edge of the soma of this neuron. Scale bar, 40 μm.

Figure 8-7. Simultaneous recording with two pipettes from the soma and dendrite of the same layer v pyramidal neuron in a rat neocortical slice. A: Simultaneous filling of the same layer V pyramidal neuron from the dendrite and the soma with different colored fluorescent dyes, Cascade blue at the soma and Lucifer yellow in the dendrite. The dendritic recording was made 190 μm from the soma. Scale bar is 40 μm. B: Subthreshold EPSPs recorded simultaneously from the dendrite and the soma of the same layer V pyramidal neuron following extracellular electrical stimulation in layer I (stimulus artifact precedes EPSPs). Dendritic recording 525 μm from the soma. C: Suprathreshold stimulation in layer I evokes an EPSP followed by action potential. Same experiment as in B. The action potential initiated by this EPSP occurs first at the soma. Simultaneous whole-cell voltage recordings were made using two microelectrode amplifiers (Axoclamp 2A, Axon Instruments, Foster City, CA). Inset represents schematic diagram of experimental arrangement. Calibration is the same for B and C.


A
B


A
B subthreshold EPSP
dendrite
soma
C suprathreshold EPSP
soma
dendrite
20 mV
10 ms




0"
5'43"
8'41"
a
b
c
9'32"
9'40"
10'19"
d
e
f
10'50"
13'22"
g
h

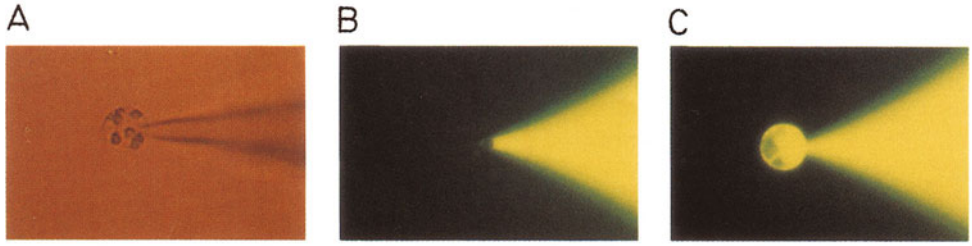

A
B
C
"cell attached"
"cell attached"
"whole-cell"
70 s

Figure 12-3. Macrosurgery releases cytoplasmic blisters from *Eremosphaera viridis.* (photograph by N. Sauer and G. Schönknecht, for details see text.)

Figure 12-4. Release of lily pollen tip protoplasts following laser microsurgery. (Reproduced from DeBoer *et al.,* 1994; for details see text.)

Figure 12-6. Equilibration of the fluorochrome Lucifer yellow with the cytoplasm of guard cells after establishment of the whole-cell configuration. (A) Cell-attached configuration transmission micrograph and (B) fluorescence micrograph with 1 μM Lucifer yellow included in the pipette solution. Note formation of an omega-shaped membrane patch in the pipette tip during the sealing process. (C) Equilibration of the pipette solution with the cytoplasm is indicated by a steady fluorescence of the cell 10 min after the establishment of the whole-cell configuration (reproduced from Marten *et al.,* 1992).