Patch-Clamp Methods and Protocols
Patch-Clamp Methods and Protocols

Edited by

Peter Molnar
and
James J. Hickman
Patch-clamp electrophysiology became commonly used since its development by Bert Sakmann and Erwin Neher in 1981. The past two decades brought major advances not only in the patch-clamp technique but in the method’s extensive applications to solve diverse scientific problems. In addition to an increase in the number of people utilizing this technique, there has been a tremendous improvement in the range of sophisticated equipment, elevating this method from the level of “wizardry” to routine usage. Recently, with the emergence of automated/parallel patch clamp, a major reorganization has taken place in the pharmacological applications of this methodology.

The goal in this volume was to summarize the typical patch-clamp applications and to assist scientists in identifying problems, which could be best addressed by this technique. In addition, this volume will provide the step-by-step procedures to perform the experiments that will answer those questions. The experiments described in this book will require a basic level of electrophysiological training. Our intention was not to teach the reader patch-clamp instrumentation and technique, as several basic and advanced level books have already been published, but to help an experimenter safely and effectively venture into new areas of electrophysiology and to routinely use the patch-clamp technique to answer scientific questions.

To enhance the enthusiasm of non-electrophysiologists toward this method, we have introduced several scientific problems as examples where combined techniques (e.g., electrophysiology + imaging or molecular biology) are most effective. Thus, encouraging scientists with diverse backgrounds to attempt to utilize patch-clamp electrophysiology.

This book was organized by the major patch-clamp application areas, namely pharmacology, physiology, and biophysics. As there are strong overlaps in the techniques utilized in these application areas, we have discussed individual techniques where they are most commonly used. Thus, avoiding any unnecessary repetition.

The first chapter provides examples and step-by-step instructions on how to use whole-cell and single-channel patch-clamp methods for testing drugs in
industrial settings. The reader will learn how to perform the electrophysiological method as well as gain insight into the drug-screening process and the standard procedures in using it for this application. A reader will also learn about how electrophysiology can be best utilized in drug research and development. The second part of this chapter provides an opportunity to compare the current automated patch-clamp systems for typical applications and thus filling a gap in this literature.

The second chapter provides a wide selection of patch-clamp applications in physiological studies. Emphasis was given to techniques where patch clamp was combined with other methodologies such as photostimulation, force measurement, polymerase chain reaction (PCR), cell patterning, or computer modeling. Also, in this chapter, two typical applications of the relatively new dynamic-clamp method are introduced. In addition, emphasis was given to the high diversity of cell types as targets of electrophysiological studies. Through examples and detailed protocols, the reader can learn how to dissect/handle/culture cell lines, primary neurons, stem cells, cardiac myocytes, and skeletal muscle cells.

The last chapter focuses on the biophysical applications of the patch-clamp method using single-channel recordings or statistical analysis of whole-cell currents to obtain parameters that describe ion channel properties or transmitter release. In this chapter, we have also included an extensive theoretical treatise concerning single-channel kinetic analysis.

We hope that many electrophysiologists and non-electrophysiologists will find this book useful in designing and performing a wide variety of patch-clamp experiments in conjunction with other state-of-the-art methodologies.

Peter Molnar
Contents

Preface .......................................................................................................................... v
Contributors .................................................................................................................. ix

PART I: PHARMACOLOGY
1. Pharmacological Analysis of Recombinant NR1a/2A and NR1a/2B NMDA Receptors Using the Whole-Cell Patch-Clamp Method
   László Fodor and József Nagy .......................................................... 3
2. Memantine as an Example of a Fast, Voltage-Dependent, Open Channel N-Methyl-d-Aspartate Receptor Blocker
   Chris G. Parsons and Kate Gilling .................................................... 15
3. Methods for Evaluation of Positive Allosteric Modulators of Glutamate AMPA Receptors
   Edward R. Siuda, Jennifer C. Quirk, and Eric S. Nisenbaum .......... 37
4. Automated Voltage-Clamp Technique
   Andrea Ghetti, António Guia, and Jia Xu .......................................... 59
5. Flip-the-Tip: Automated Patch Clamping Based on Glass Electrodes
   Michael Fejtl, Uwe Czubayko, Alexander Hümmcr, Tobias Krauter, and Albrecht Lepple-Wienhues .................. 71
6. The Roboocyte: Automated Electrophysiology Based on Xenopus Oocytes
   Christine Leisgen, Mike Kuester, and Christoph Methfessel .......... 87

PART II: PHYSIOLOGY
7. Infrared-Guided Laser Stimulation as a Tool for Elucidating the Synaptic Site of Expression of Long-Term Synaptic Plasticity
   Gerhard Rammes, Matthias Eder, Walter Zieglgänsberger, and Hans-Ulrich Dodt ........................................ 113
8. Single-Cell RT–PCR, a Technique to Decipher the Electrical, Anatomical, and Genetic Determinants of Neuronal Diversity
   Maria Toledo-Rodriguez and Henry Markram .................................. 123
<table>
<thead>
<tr>
<th>No.</th>
<th>Title</th>
<th>Authors/Editors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.</td>
<td>Mechanosensitive Ion Channels Investigated Simultaneously by Scanning Probe Microscopy and Patch Clamp</td>
<td>Matthias G. Langer</td>
<td>141</td>
</tr>
<tr>
<td>10.</td>
<td>Synaptic Connectivity in Engineered Neuronal Networks</td>
<td>Peter Molnar, Jung-Fong Kang, Neelima Bhargava, Mainak Das, and James J. Hickman</td>
<td>165</td>
</tr>
<tr>
<td>11.</td>
<td>Modeling of Action Potential Generation in NG108-15 Cells</td>
<td>Peter Molnar and James J. Hickman</td>
<td>175</td>
</tr>
<tr>
<td>12.</td>
<td>Whole-Cell Voltage Clamp on Skeletal Muscle Fibers With the Silicone-Clamp Technique</td>
<td>Sandrine Pouvreau, Claude Collet, Bruno Allard, and Vincent Jacquemond</td>
<td>185</td>
</tr>
<tr>
<td>13.</td>
<td>Determination of Channel Properties at the Unitary Level in Adult Mammalian Isolated Cardiomyocytes</td>
<td>Romain Guinamard</td>
<td>195</td>
</tr>
<tr>
<td>14.</td>
<td>Electrophysiological Properties of Embryonic Stem Cells During Differentiation Into Cardiomyocyte-Like Cell Types</td>
<td>Antoni C. G. van Ginneken and Arnoud C. Fijnvandraat</td>
<td>211</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Part III: Biophysics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>Principles of Single-Channel Kinetic Analysis</td>
<td>Feng Qin</td>
<td>253</td>
</tr>
<tr>
<td>18.</td>
<td>Use of <em>Xenopus</em> Oocytes to Measure Ionic Selectivity of Pore-Forming Peptides and Ion Channels</td>
<td>Thierry Cens and Pierre Charnet</td>
<td>287</td>
</tr>
<tr>
<td>19.</td>
<td>Estimation of Quantal Parameters With Multiple-Probability Fluctuation Analysis</td>
<td>Chiara Saviane and R. Angus Silver</td>
<td>303</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>319</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Contributors

Bruno Allard • Physiologie Intégrative Cellulaire et Moléculaire, Université Claude Bernard Lyon 1, France
Géza Berecki • Department of Experimental Cardiology, Academic Medical Center, University of Amsterdam, The Netherlands
Jonathan Bettencourt • Department of Biomedical Engineering, Center for BioDynamics, Center for Memory and Brain, Boston University, Boston, MA, and Department of Physiology and Biophysics, Weill Medical College of Cornell University, New York, NY
Neelima Bhargava • Nanoscience Technology Center, University of Central Florida, Orlando, FL
Thierry Cens • CRBM, CNRS, France
Pierre Charnet • CRBM, CNRS, France
Claude Collet • Laboratoire de Toxicologie Environnementale, Ecologie des Invertébrés, France
Uwe Czubayko • Flyion GmbH, Germany
Mainak Das • Nanoscience Technology Center, University of Central Florida, Orlando, FL
Hans-Ulrich Dodt • Max-Planck-Institute of Psychiatry, Germany
Alan D. Dorval II • Department of Biomedical Engineering, Center for BioDynamics, Center for Memory and Brain, Boston University, Boston, MA, and Department of Biomedical Engineering, Duke University, Durham, NC
Mathias Eder • Max-Planck-Institute of Psychiatry, Germany
Michael Feitl • Flyion GmbH, Germany
Arnaud C. Fijnvandraat • Department of Experimental Cardiology and Experimental and Molecular Cardiology Group, Academic Medical Center, University of Amsterdam, The Netherlands
László Fodor • Pharmacology and Drug Safety Research, Gedeon Richter Ltd., Hungary
Andrea Ghetti • AVIVA Biosciences Corp, San Diego, CA
Kate Gilling • Preclinical Research & Development, Merz Pharmaceuticals GmbH, Germany
Antoni C. G. van Ginneken • Department of Experimental Cardiology and Experimental and Molecular Cardiology Group, Academic Medical Center, University of Amsterdam, The Netherlands
António Guia • AVIVA Biosciences, San Diego, CA
Romain Guinamard • CNRS, UMR 6187, Université de Poitiers, France
James J. Hickman • Nanoscience Technology Center, University of Central Florida, Orlando, FL
Alexander Hümmer • Flyion GmbH, Germany
Vincent Jacquemond • Physiologie Intégrative Cellulaire et Moléculaire, Université Claude Bernard Lyon 1, France
Jung-Fong Kang • Nanoscience Technology Center, University of Central Florida, Orlando, FL
Tobias Krauter • Flyion GmbH, Germany
Mike Kuester • Bayer Technology Services GmBH, Germany
Matthias G. Langer • University of Ulm, Germany
Christine Leisgen • Multi Channel Systems MCS GmbH, Germany
Albrecht Leppe-Wienhues • Flyion GmbH, Germany
Henry Markram • Brain and Mind Institute, EPFL, Switzerland
Christoph Methfessel • Bayer Technology Services GmBH, Germany
Peter Molnar • Nanoscience Technology Center, University of Central Florida, Orlando, FL
József Nagy • Pharmacology and Drug Safety Research, Gedeon Richter Ltd., Hungary
Theoden I. Netoff • Department of Biomedical Engineering, University of Minnesota Minneapolis, MN
Eric S. Nisenbaum • Neuroscience Division, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN
Chris G. Parsons • Head In Vitro Pharmacology, Preclinical Research & Development, Merz Pharmaceuticals GmbH, Germany
Sandrine Pouvreau • Physiologie Intégrative Cellulaire et Moléculaire, Université Claude Bernard Lyon 1, France
Feng Qin • Department of Physiology and Biophysics, State University of New York at Buffalo, Buffalo, NY
Jennifer C. Quirk • Neuroscience Division, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN
Gerhard Rammes • Max-Planck-Institute of Psychiatry and Technical University, Klinikum Rechts der Isar, Germany
Chiara Saviane • University College London, London, UK
Robin Angus Silver • University College London, London, UK
Contributors

Edward R. Siuda • Neuroscience Division, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN
Maria Toledo-Rodriguez • Brain and Mind Institute, EPFL, Switzerland
John A. White • Department of Biomedical Engineering, Center for BioDynamics, Center for Memory and Brain, Boston University, Boston, MA
Ronald Wilders • Department of Physiology, Academic Medical Center, University of Amsterdam, The Netherlands
Jia Xu • AVIVA Biosciences, San Diego, CA
Jan G. Zegers • Department of Physiology, Academic Medical Center, University of Amsterdam, The Netherlands
Walter Zieglegänsberger • Max-Planck-Institute of Psychiatry, Germany