

Neuroanatomical Tract-Tracing 3

Molecules, Neurons, and Systems

Neuroanatomical Tract-Tracing 3

Molecules, Neurons, and Systems

Edited by

Laszlo Zaborszky
Rutgers University
Newark, NJ, USA

Floris G. Wouterlood
Vrije University
Amsterdam, The Netherlands

José Luis Lanciego
University of Navarra
Pamplona, Spain

 Springer

Laszlo Zaborszky
Center for Molecular and
Behavioral Neuroscience
Rutgers University
Newark, NJ, USA

Floris G. Wouterlood
Department of Anatomy
Vrije University Medical Center
Amsterdam, The Netherlands

José Luis Lanciego
Neurosciences Division
Center for Applied
Medical Research
(CIMA)
University of Navarra
Pamplona, Spain

Cover illustration: Reconstruction of functional connectivity between neurons based on the temporal coherence of spiking activity recorded extracellularly from the somatosensory cortex of a rat. See Fig. 20.8 on page 666.

Library of Congress Control Number: to come

ISBN-10: 0-387-29078-8
ISBN-13: 978-0387-29078-2

Printed on acid-free paper.

© 2006 Springer Science+Business Media, Inc.

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Springer Science+Business Media, Inc., 233 Spring Street, New York, NY 10013, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.

The use in this publication of trade names, trademarks, service marks, and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

Printed in the United States of America. (TB/KYO)

9 8 7 6 5 4 3 2 1

springer.com

To Sarah Z for her 18th birthday

Contents

Preface	ix
1. Short Retrospection <i>Lennart Heimer</i>	1
2. Preembedding Immunolectron Microscopy: Applications for Studies of the Nervous System <i>Susan R. Sesack, LeeAnn H. Miner, and Natalia Omelchenko</i>	6
3. Postembedding Immunogold Cytochemistry of Membrane Molecules and Amino Acid Transmitters in the Central Nervous System <i>Thomas Misje Mathiisen, Erlend Arnulf Nagelhus, Bahareh Jouleh, Reidun Torp, Didrik Sølve Frydenlund, Maria-Niki Mylonakou, Mahmood Amiry-Moghaddam, Luciene Covolan, Jo Kristian Utvik, Björg Riber, Karen Marie Gujord, Jorunn Knutsen, Øivind Skare, Petter Laake, Svend Davanger, Finn-Mogens Haug, Eric Rinvik, and Ole Petter Ottersen</i>	72
4. Cell and Tissue Microdissection in Combination with Genomic and Proteomic Applications <i>Stephen D. Ginsberg, Scott E. Hemby, Elliott J. Mufson, and Lee J. Martin</i>	109
5. Molecules and Membrane Activity: Single-Cell RT-PCR and Patch-Clamp Recording from Central Neurons <i>William H. Griffith, Sun-Ho Han, Brian A. McCool, and David Murchison</i>	142
6. Merging Structure and Function: Combination of In Vivo Extracellular and Intracellular Electrophysiological Recordings with Neuroanatomical Techniques <i>Attila Sík</i>	175
7. Juxtacellular Labeling of Individual Neurons In Vivo: From Electrophysiology to Synaptology <i>Alvaro Duque and Laszlo Zaborszky</i>	197

8. Nonradioactive In Situ Hybridization in Combination with Tract-Tracing	237
<i>Ruth L. Stornetta and Patrice G. Guyenet</i>	
9. Viral Tracers for the Analysis of Neural Circuits	263
<i>Joel C. Geerling, Thomas C. Mettenleiter, and Arthur D. Loewy</i>	
10. Dextran Amines: Versatile Tools for Anterograde and Retrograde Studies of Nervous System Connectivity	304
<i>Anton Reiner and Marcia G. Honig</i>	
11. Multiple Neuroanatomical Tract-Tracing: Approaches for Multiple Tract-Tracing	336
<i>José L. Lanciego and Floris G. Wouterlood</i>	
12. Tract-Tracing in Developing Systems and in Postmortem Human Material Using Carbocyanine Dyes	366
<i>Zoltán Molnár, Daniel Blakey, Irina Bystron, and Rosalind S. E. Carney</i>	
13. Combined Fluorescence Methods to Determine Synapses in the Light Microscope: Multilabel Confocal Laser Scanning Microscopy	394
<i>Floris G. Wouterlood</i>	
14. Advances in Understanding Cortical Function Through Combined Voltage-Sensitive Dye Imaging, Whole-Cell Recordings, and Analysis of Cellular Morphology	436
<i>Carl C. H. Petersen</i>	
15. From Dendrites to Networks: Optically Probing the Living Brain Slice and Using Principal Component Analysis to Characterize Neuronal Morphology	452
<i>Jesse H. Goldberg, Farid-Hamzei-Sichani, Jason MacLean, Gabor Tamas, Rochelle Urban, and Rafael Yuste</i>	
16. Stereology of Neural Connections: An Overview	477
<i>Carlos Avendaño</i>	
17. Three-Dimensional Computerized Reconstruction from Serial Sections: Cell Populations, Regions, and Whole Brain	530
<i>Jan G. Bjaalie and Trygve B. Leergaard</i>	

viii CONTENTS

18. Atlases of the Human Brain: Tools for Functional Neuroimaging	566
<i>Katrin Amunts and Karl Zilles</i>	
19. Neuron and Network Modeling	604
<i>Giorgio A. Ascoli and Ruggero Scorcioni</i>	
20. Functional Connectivity of the Brain: Reconstruction from Static and Dynamic Data	631
<i>Zoltan Nadasdy, Gyorgy Buzsaki, and Laszlo Zaborszky</i>	

Preface

Between the first edition of *Neuroanatomical Tract-Tracing Methods* in 1981 and the current, third edition, neuroscience has witnessed a total transition into the information age. Scientists, whether they wanted it or not, have turned digital. Today, everyone is linked up with worldwide computer communication networks, with local and worldwide digital environments offering vastly increased speed and accuracy of data acquisition and processing. Communication and exchange of information between scientists worldwide is a matter of seconds. The electronic dissemination of research data has become routine. Publication of scientific results has changed from the typewritten manuscript to electronic online submission. Search engines, PubMed-like services, and electronic notification and delivery services are making life more convenient for scientists. *What is not on the Web does not exist.* Do we still need books?

We think positively about books. In the first place, it is common sense to have at hand a printed technical protocol in the setting of a laboratory engaged in experimental neuroscience. Although the workbench protocol does not necessarily have to be a book, it is nonetheless helpful to have a book at hand that not only provides the technical protocol, but also explains why the protocol is designed as it is, what the alternatives are, and what their consequences are. The Web is a wonderful yet particularly fluid medium in which things change very quickly. Data that were here today are gone tomorrow. A book has a longer time constant, which sometimes is beneficial.

The first two editions of the book (Heimer and Robards, 1981; Heimer and Zaborszky, 1989—both published by Plenum Press) had a tremendous impact on neuroscience. They are still among the frequently consulted books in the laboratory. We feel that the moment has arrived to pursue a third, thoroughly updated version of this landmark book, in order to continue the line originated by *Neuroanatomical Tract-Tracing Methods*. The target audience remains the graduate students and young investigators working in the laboratory, seeking fast, complete, up-to-date, and immediately applicable information about techniques, written by acknowledged experts in the field.

Since the last edition, several methods that were in their infancy 15 years ago have become routine, older methods have experienced a renaissance, and newly emerging techniques need validation. Molecular techniques, such as genomics and proteomics, have become established methods, which allow for the study of gene expression of recorded and traced neurons (chapters by Ginsberg, Griffith, Stornetta, and their colleagues). The simultaneous

x PREFACE

development of new fluorescence probes, single- and multiphoton confocal laser scanning microscopes, and vastly increased computer processing power have contributed to a renaissance of fluorescence methods (chapters by Lanciego, Molnar, Reiner, and Wouterlood). In vivo tractography as well as structural and functional imaging techniques allow for the study of the living human brain with a degree of detail never dreamed possible (chapter by Amunts and Zilles). Immunocytochemistry using pre- and postembedding electron microscopy for identifying neuroactive substances still remains an art which has to be tailored to one's needs (chapters by Sesack, Ottersen, and their coworkers). Viral tracers for the analysis of neural circuits have become established in some laboratories (chapter by Geerling and his colleagues). Several chapters briefly discuss how databases help in acquisition, analysis, modeling, and integration of complex cross-scale data sets (chapters by Zilles, Bjaalie, and Nadasdy). Readers interested in these topics are referred to a recently published textbook edited by Koslow and Subramaniam (2005) that summarizes efforts in this field led by the Human Brain Project of the National Institutes of Health.

As neuroscience research progresses, we witness drastic changes in how methods are used. The previous editions of this series reflected the reductionist approach to study neuroscience which was characteristic for the previous century. Even in the second edition, only 2 out of 13 chapters combined techniques that crossed the traditional borders of anatomy and physiology. In the present edition, most of the chapters describe methods, which allow for the integration of molecular, cellular, and system level data, reflecting a holistic-integrative approach to neuroscience in the twenty-first century. Specifically, using sophisticated combinations of tracing methods, the molecular and genetic identity of a neuron (chapters by Ginsberg and his colleagues), as well as the synaptology of any circuitry can be accurately determined (chapter by Sesack and her coworkers). Using extracellular, juxtacellular, and intracellular recordings (chapters by Duque and Zaborszky, and Sik), anatomical features can be correlated with electroencephalographic (EEG), multiunit activity (MUA), local field potentials, and intrinsic membrane characteristics. Recent advances in voltage-sensitive dye imaging (chapter by Petersen) and two-photon calcium imaging (chapter by Goldberg et al.) are promising techniques for studying the spatiotemporal dynamics of hundreds of neurons in the living brain. Sophisticated statistical designs (Avendaño) and expanding computational approaches have the potential to capture full three-dimensional (3D) relations of neuronal and architectonic features of entire brain systems (chapters by Ascoli, Bjaalie, and their colleagues). The last chapter (by Nadasdy *et al.*) predicts that within the next 10 years the complete 3D vectorial database of the rat brain will be available to address specific questions about hidden organization principles of the nervous system. However, these authors also address the gap that exists in our understanding between "structural" and "functional" connectivity (e.g., Friston *et al.*, 1993). We hope that students of this book will bridge this gap eventually, leading to a better understanding of

how the human brain functions in health, aging, and disease. This is our ultimate goal.

ACKNOWLEDGMENT

It has been an honor as well as a pleasure to select contributors for the current edition of *Neuroanatomical Tract-Tracing Methods* and to produce with them this book. However, this book would never have been written without several generations of scientists designing and optimizing the methods discussed in the chapters. Among all those whose shared legacy is the current technological standard, we would like to specially mention the recently deceased **Sanford Palay** (Sandy) (1992), who saw the first synapse using electron microscopy and **Theodor Blackstad** (see, e.g., Blackstad and Bjaalie, 1988) whose contribution was instrumental to computational neuroanatomy as we understand it today. We had the great privilege to work or interact with them. However, we learned the most from our mentors, **Lennart Heimer** and **Enrico Mugnaini**, pioneers in tract-tracing and cellular neuroscience. They were not only mentors and teachers, but friends as well. We dedicate this book to them.

We thank the authors for their contributions and patience during the somewhat lengthy editorial process. They exerted great effort in writing their chapters, and also provided essential feedback by cross-reviewing each manuscript. We are also indebted to Drs. James Tepper (Rutgers University), Harry Uylings (The Netherlands Brain Research Institute), and Rolf Kötter (C. and O. Vogt Brain Research Institute, Düsseldorf) who as external reviewers read earlier versions of some of the chapters. We have the fortune to have on our side Kathleen Lyon, Senior Biosciences Editor of Springer, who spearheaded this edition with great enthusiasm. Last, but not least, we would like to acknowledge Professors Ian Creese and Paula Tallal, codirectors of the Center for Molecular and Behavioral Neuroscience, Rutgers University, for encouragement. The National Institutes of Health (L.Z.) gave generous financial support over many years. Elizabeth Hur, helping the editorial process at Rutgers University, was the first Graduate Student who read and benefited from this book. We hope many will follow.

Laszlo Zaborszky
Floris G Wouterlood
Jose L Lanciego

REFERENCES

- Blackstad, T. W., and Bjaalie, J. G., 1988, Computer programs for neuroanatomy: three-dimensional reconstruction and analysis of populations of cortical neurons and other bodies with a laminar distribution. *Comput. Biol. Med.* **18**:321–340.

xii PREFACE

- Friston, K. J., Frith, C. D., Liddle, P. F., and Frackowiak, R. S., 1993, Functional connectivity: the principal-component analysis of large (PET) data sets. *J. Cereb. Blood Flow Metab.* **13**:5–14.
- Heimer, L., and Robards, M., 1981, *Neuroanatomical Tract-Tracing Methods*, New York: Plenum Press, p. 567.
- Heimer, L., and Zaborszky, L., 1989, *Neuroanatomical Tract-Tracing Methods 2. Recent Progress*, New York: Plenum Press, p. 408.
- Koslow, S. H., and Subramaniam, S., 2005, *Databasing the Brain: From Data to Knowledge*, Hoboken: Wiley-Liss, p. 466.
- Palay, S. L., 1992, A concatenation of accidents, In: Samson, F. S., Adelman, G. (eds.), *The Neurosciences: Paths of Discovery*, II, Boston: Birkhauser, pp. 191–212.