

# METHODS IN MOLECULAR BIOLOGY™

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# **Neuropeptides**

## **Methods and Protocols**

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## Preface

The term *neuropeptide* was originally coined to indicate small protein molecules that are contained in neurons. In the late 1970s and the 1980s of the last century, several tens of neuropeptides were localized by immunocytochemistry to discrete cell populations of the central and peripheral nervous system, and the concept of *chemical neuroanatomy*, originally developed by Tomas Hökfelt and coworkers, entered the scene of neurobiology. Since then, the field of neuropeptide biology has dramatically widened, and today the ultimate frontiers in neuropeptide research lie in the development of pharmacologically active compounds that are capable of crossing the blood–brain barrier to exert their biological role(s) *in vivo* and in the construction of genetic vectors to be employed in gene therapy.

This book represents a readily reproducible collection of established and emerging techniques for neuropeptide research. Such a collection is preceded by a general introductory chapter (Chapter 1) that discusses a series of new concepts leading to a broader neuropeptide definition in light of the huge amount of data accumulated after more than half a century of neuropeptide research.

The methods presented include immunocytochemical localization, biochemical characterization, functional analysis, development and production of genetic probes, and the design of neuropeptide derivatives for cellular neurobiology as well as the potential therapeutic applications.

As a general indication to the readers, Chapters 2–10 are focused on a series of techniques for localization studies. They cover a broad range of protocols, such as the immunocytochemical detection of neuropeptides in nonmammalian vertebrates together with a detailed description of procedures for anesthesia and tissue preparations in these species (Chapter 2); the combined neuropeptide/receptor localization at the light and transmission electron microscope for connectivity studies (Chapter 3); the analysis of neuropeptide genes' transcription by localization of pre-mRNA (Chapter 6) or mRNA/microRNA with *in situ* hybridization (Chapter 4), *in situ* PCR (Chapter 5), and laser capture/microdissection (Chapter 7); the visualization *in vivo* of neuropeptide secretion (Chapter 8) and translocation across the plasma membrane (Chapter 9); and the functional analysis of neuropeptide interactions *in vitro* with cells of the immune system (Chapter 10).

Chapter 11 describes a series of electrophysiological protocols for functional studies *in vitro* and *in vivo*.

Chapters 12–19 are devoted to biochemical/molecular biology techniques, ranging from radioimmunoassay (Chapter 12) to neuropeptidomics employing reverse-phase HPLC (Chapter 13) or mass spectrometry (Chapter 14), RNA analysis by suppression subtractive hybridization (Chapter 15), determination of neuropeptide release *in vivo* by microdialysis (Chapter 16) or antibody microprobes (Chapter 17), and measurement of neuropeptidases (Chapter 18) and neuropeptide autoantibody levels (Chapter 19) in biological fluids.

Chapters 20–24 deal with a number of techniques developed to optimize neuropeptide administration to central neurons or to interfere with biological effects *in vivo*. These procedures include the intranasal delivery of neuropeptides (Chapter 20), the development of

neuropeptide pro-drugs (Chapter 21), the use of phosphorothioate oligodeoxynucleotides that are capable of crossing the blood–brain barrier to knock down neuropeptides in the CNS (Chapter 22), the development of liposome-encapsulated neuropeptides for assessing the chronic actions of physiologically short-lived molecules (Chapter 23), the construction of recombinant adeno-associated viral vectors that can be used to locally or systemically enhance or silence neuropeptide gene expression (Chapter 24).

Finally, Chapter 25 describes a calcium mobilization assay in mammalian cells to identify novel G-protein-coupled receptor family members that transduce the neuropeptide signals.

All scientists who have excellently contributed to this book have a direct experience in one or more fields of neuropeptide research. I am very much indebted to all of them for their successful effort in emphasizing the description of the more common pitfalls in the techniques that they have described and of the hints to reduce the possibility of failure for beginners.

The collection of protocols that forms this book is surely not exhaustive of the wide range of approaches that today can be employed in top level neuropeptide research. Yet it is intended for a large audience of scientists, including histologists, biochemists, cellular and molecular biologists, and electrophysiologists that are currently active in the field or are willing to enter such an exciting and still expanding area of neurobiology.

*Grugliasco, TO, Italy*

*Adalberto Merighi*

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## Contents

<i>Preface</i> . . . . .	<i>v</i>
<i>Contributors</i> . . . . .	<i>ix</i>
1 What Are Neuropeptides? . . . . . <i>J. Peter H. Burbach</i>	1
2 Neuropeptide Localization in Nonmammalian Vertebrates . . . . . <i>Paolo de Girolamo and Carla Lucini</i>	37
3 Combined Light and Electron Microscopic Visualization of Neuropeptides and Their Receptors in Central Neurons . . . . . <i>Chiara Salio, Laura Lossi, and Adalberto Merighi</i>	57
4 Neuropeptide RNA Localization in Tissue Sections . . . . . <i>Marc Landry, Shérine Abdel Salam, and Marie Moftah</i>	73
5 Intron-Specific Neuropeptide Probes . . . . . <i>Harold Gainer, Todd A. Ponzio, Chunmei Yue, and Makoto Kawasaki</i>	89
6 Direct In Situ RT-PCR . . . . . <i>Laura Lossi, Graziana Gambino, Chiara Salio, and Adalberto Merighi</i>	111
7 Laser Capture Microdissection and Quantitative-PCR Analysis . . . . . <i>Sarah J. Paulsen and Leif K. Larsen</i>	127
8 Visualization of Peptide Secretory Vesicles in Living Nerve Cells . . . . . <i>Joshua J. Park and Y. Peng Lob</i>	137
9 Fluorescence Imaging with Single-Molecule Sensitivity and Fluorescence Correlation Spectroscopy of Cell-Penetrating Neuropeptides . . . . . <i>Vladana Vukojević, Astrid Gräslund, and Georgy Bakalkin</i>	147
10 Analysis of Neuroimmune Interactions by an In Vitro Coculture Approach . . . . . <i>Tadahide Furuno and Mamoru Nakanishi</i>	171
11 Electrophysiology . . . . . <i>Zhi-Qing David Xu</i>	181
12 Localization of Neuropeptides by Radioimmunoassay . . . . . <i>Fred Nyberg and Mathias Hallberg</i>	191
13 Reversed-Phase HPLC and Hyphenated Analytical Strategies for Peptidomics . . . . . <i>Anne-Marie Hesse, Segá Ndiaye, and Joelle Vinh</i>	203
14 Neuropeptidomics: Mass Spectrometry-Based Qualitative and Quantitative Analysis . . . . . <i>Ping Yin, Xiaowen Hou, Elena V. Romanova, and Jonathan V. Sweedler</i>	223
15 Suppression Subtractive Hybridization . . . . . <i>Mohamed T. Ghorbel and David Murphy</i>	237

16	Neuropeptide Microdialysis in Free-Moving Animals . . . . .	261
	<i>Tetsuya Kushikata and Kazuyoshi Hirota</i>	
17	Antibody Microprobes for Detecting Neuropeptide Release . . . . .	271
	<i>Rebecca J. Steagall, Carole A. Williams, and Arthur W. Duggan</i>	
18	Neuropeptidases . . . . .	287
	<i>Manuel Ramírez, Isabel Prieto, Inmaculada Banegas, Ana B. Segarra, and Francisco Alba</i>	
19	Neuropeptide Autoantibodies Assay . . . . .	295
	<i>Sergueï O. Fetissov</i>	
20	Intranasal Delivery of Neuropeptides . . . . .	303
	<i>Michael C. Veronesi, Daniel J. Kubek, and Michael J. Kubek</i>	
21	Prodrug Design for Brain Delivery of Small- and Medium-Sized Neuropeptides . . . . .	313
	<i>Katalin Prokai-Tatrai and Laszlo Prokai</i>	
22	Measurement of Phosphorothioate Oligodeoxynucleotide Antisense Transport Across the Blood–Brain Barrier . . . . .	337
	<i>William A. Banks</i>	
23	Liposome-Encapsulated Neuropeptides for Site-Specific Microinjection . . . . .	343
	<i>Frédéric Frézard, Robson A.S. dos Santos, and Marco A.P. Fontes</i>	
24	Recombinant Adeno-Associated Viral Vectors . . . . .	357
	<i>Marijke W.A. de Backer, Keith M. Garner, Mieneke C.M. Luijendijk, and Roger A.H. Adan</i>	
25	Deorphanizing G Protein-Coupled Receptors by a Calcium Mobilization Assay . . . . .	377
	<i>Isabel Beets, Marleen Lindemans, Tom Janssen, and Peter Verleyen</i>	
	<i>Index</i> . . . . .	393



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